pH-RESPONSIVE HYDROGEL-BASED CHEMOMECHANICAL SENSORS DESIGNED FOR DISPOSABLE BIOREACTOR APPLICATIONS

by

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STATEMENT OF DISSERTATION APPROVAL

The following faculty members served as the supervisory committee chair and members for the dissertation of Jeffrey Bates.

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ABSTRACT

Stimuli-responsive hydrogels are called "smart" materials because they autonomously respond to environmental stimuli. For example, pH-responsive hydrogels swell at lower pH levels and deswell as the pH increases. Hydrogel-based sensors could prove beneficial for providing continuous monitoring of bioreactors. The motivation of this project is to create a hydrogel-based sensor that can be used for bioreactor monitoring to help researchers monitor bioreactor conditions. The magnitude of the swelling/deswelling behavior can be measured by placing a sample of the hydrogel in a piezoresistive sensor. The degree of swelling/deswelling is directly proportional to the change in pH of the aqueous solution in which it is placed. In this project, an initial characterization of the hydrogel response was performed, followed by an analysis of the hydrogel components and optimization of the hydrogel response based on those components. The longevity of the hydrogel response was tested in terms of shelf life and response after multicycle testing. A hydrogel sample was then synthesized in situ in a microsensor and tested to determine the ability to transport hydrogels and how the miniaturization of the sensor may affect the stimuli response. In all experiments, the response time and magnitude results were compared to determine the effect of the noted changes on the kinetics of the swelling behavior of the material in order to find the optimal composition, thickness, and device specifications that will yield the desired response time and sensitivity.

For Emily

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CHAPTER 1

INTRODUCTION

1.1 Motivation

1.1.1 Overview of Bioreactors

Bioreactors are systems designed to support an environment that is biologically active. The processes that occur within a bioreactor may be aerobic or anaerobic. Bioreactors are used in biochemical engineering processes, tissue engineering, and generating cell cultures. In all cases, cells, tissues, and other biological chemicals are able to perform at a very high success rate because of the optimum conditions and controlled environment of a bioreactor [4]. Therefore, optimal biological processing and growth depends on the tight control of the system. In order to maintain optimum conditions, certain environmental factors must be closely monitored [5]. The successful operation of bioreactors relies upon the monitoring and control of closely monitored factors that affect the behavior of the system. Factors include oxygen, nitrogen, and carbon dioxide flow rates; temperature; pH dissolved oxygen and even circulation rate [6]. Furthermore, the sensing mechanisms cannot react with the internal environment and they cannot compromise the sterility. If monitored conditions are altered even slightly,

contamination may occur. Contamination affects the sterility and efficiency of the bioreactor environment and could result in the loss of thousands of dollars [7-9].

The pH of a bioreactor is one of the closely monitored environmental factors because even small changes in pH can influence the synthesis of biological matrix molecules, including proteins and other tissue scaffold materials. The change in systemic pH levels may be an indicator of perturbations in metabolic activity as a result of drug or toxin effect [10]. However, it may also be an indicator of anaerobic or anoxic processes in the bioreactor system. The pH level of the system responds to microbial reactions. An increased pH is an indicator of ammonification and denitrification, while a decreased pH can indicate nitrification [7].

For bioreactor processes, it is important to maintain continuous monitoring of all factors. If conditions change, then modifications need to be made to controls to compensate. Small changes must be corrected quickly in order to maintain a high bioreactor success rate. In addition, continuous monitoring is important because if sensors are placed into the bioreactor and removed repeatedly throughout the course of an experiment, contaminants are introduced each time the monitoring devices are reintroduced to the bioreactor system [4-17].

1.2 Background

1.2.1 Hydrogels

Hydrogels are super absorbent network polymers consisting of three-dimensional structures that can absorb and retain water and other aqueous fluids while maintaining the original structure. Hydrogels are made of water soluble monomer backbone molecules

with a cross-linking molecule selected for either physical or chemical properties. Physical cross-linking is created by an interaction of the hydrogel matrix with the analyte molecule. Chemical cross-linking is created with a permanent junction, usually a vinyl group on both ends of a longer chain molecule (see Figure 1.1) [18-29].

However, while many hydrogels are cross-linked either physically or chemically, some hydrogels may consist of entangled fibers or even colloidal assemblies. Hydrogels are elastic networks with interstitial spaces that may contain as much as 90-99 weight % of water, therefore absorbing water and other fluids up to 10-20 times the molecular

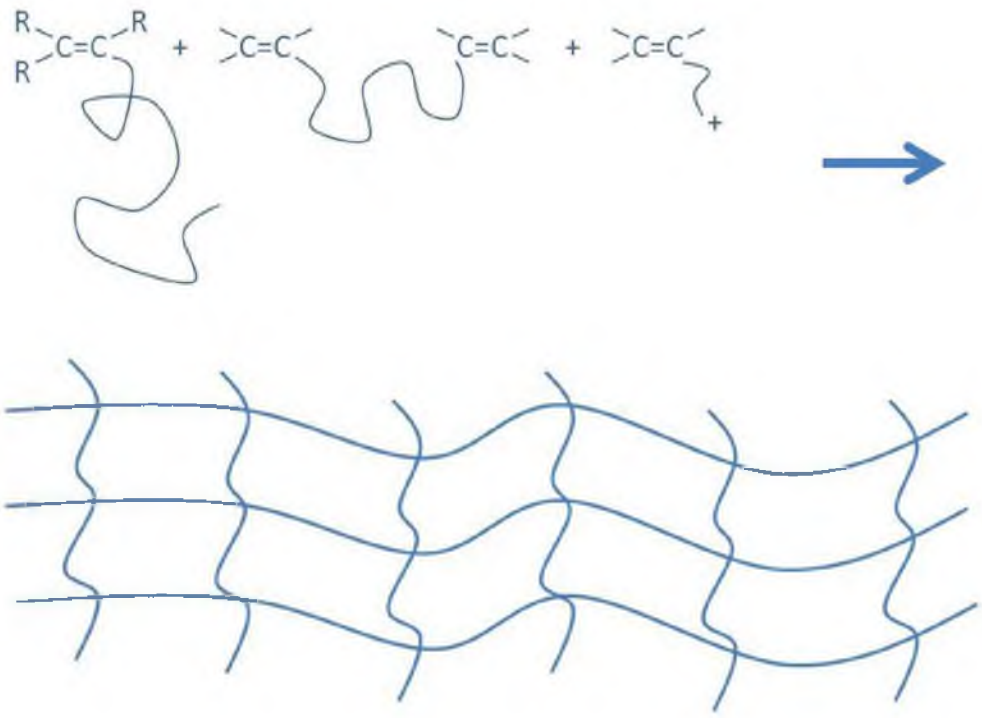


Figure 1.1. Hydrogel backbone molecules are combined with cross-linking molecules with varying lengths of cross-linking chain molecules and molecules that may be functionalized and polymerized with free radical polymerization to create chemically cross-linked, network polymers that respond to changes in environmental conditions.

weight of the original network. Due to the network structure, hydrogels resemble the highly hydrated state of natural tissues, which make them good candidates for both tissue engineering and drug delivery [18-29]. Furthermore, hydrogels have ideal mechanical and chemical properties for use as biosensors. The extreme porosity of the matrix permits rapid analyte diffusion, which takes advantage of the entire three-dimensional structure [18-83].

1.2.2 Stimuli Response of Hydrogels

Hydrogels are good candidates in biomedical applications because of their response to changes in the local environment. Hydrogels may swell or deswell depending on the conditions of the surrounding aqueous media. The swelling response is currently being harnessed in biological sensing applications for the detection of both analytes in solutions and biological compounds [18-29]. Hydrogels are known to respond to changes in pH, glucose concentration, ionic strength, temperature, electric field, solvent composition, and pressure (see Figure 1.2).

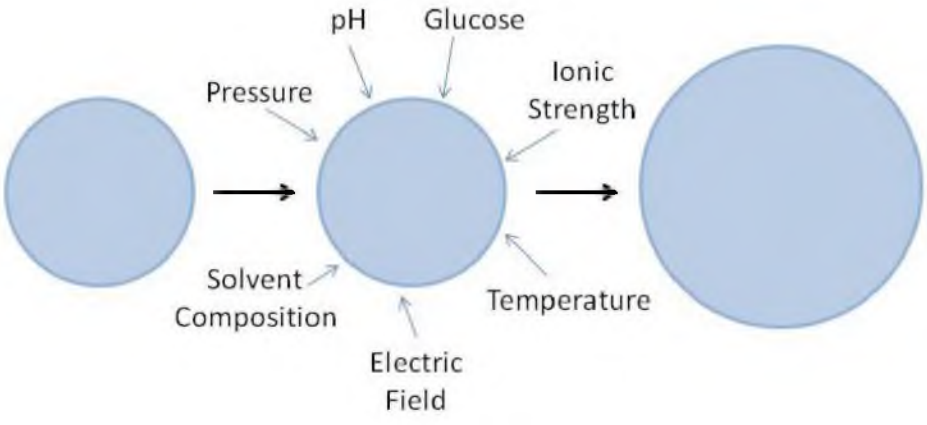


Figure 1.2. Hydrogels swell and deswell based on the change in environmental conditions, which makes them good candidates for sensing applications.

The method of detection is dependent upon the structure of the hydrogel matrix. In order for hydrogels to elicit a response to the surrounding media solution, they must include monomers with side chains that may be functionalized. The matrix functional modification may occur as a result of chemical bonding or ionic interactions. Hydrogels that utilize ionic interactions have the ability to change the charge on the side chains in response to surrounding environmental conditions (see Figure 1.3) [54-74].

As the charge changes within the hydrogel matrix, the hydrogel swells due to intermolecular electrostatic repulsion [40-42]. The swelling ratio of a hydrogel response is calculated with the following equation:

$$Qs = \frac{Ws - W_o}{W_o} \tag{1.1}$$

In equation 1.1, Q_s represents the swelling ratio, W_s represents the weight of the swollen gel, and W_o represents the weight of the original gel.

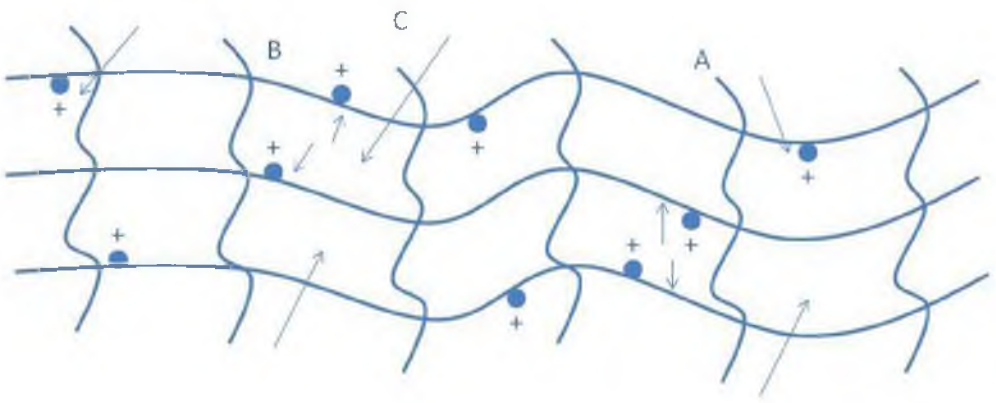


Figure 1.3. Ions pass into the hydrogel matrix and charge the side chains (A). Charges on the side chains repel each other causing the hydrogel to swell (B). Water enters the hydrogel matrix causing water absorption into the matrix (C).

1.2.3 Thermodynamics

Mathematical models have been proposed for determining the equilibrium state of hydrogels. Each of these models stems from the equation for calculating the change in Gibbs free energy of mixing [40-42]:

$$\Delta G_m = \Delta H_m - T\Delta S_m \tag{1.2}$$

In equation 1.2, ΔG_m represents the change in Gibbs free energy of the system, ΔH_m represents the change in enthalpy, T is the temperature, and ΔS_m represents the change in entropy after mixing. The change in free energy can be applied to hydrogel swelling by defining the total change in free energy ΔG_{tot} as hydrogel swelling comes to equilibrium, or as the chemical potential of each species in a solution becomes equal in coexisting phases. For hydrogels, there are two components, negative and positive, that affect change to the total change in free energy. The negative component occurs when the polymer segments are mixed with water, ΔG_{mix}, and the positive component is associated with the change in entropy that occurs as a result of polymer matrix stretching, ΔG_{el}. For hydrogels with a polyelectrolyte response, there is an additional component that must be considered, which occurs as a result of water mixing with ions within the hydrogel matrix, ΔG_{ion}. Each of these terms is independent, and the resulting equation is represented as (1.3):

$$\Delta G_{tot} = \Delta G_{mix} + \Delta G_{el} + \Delta G_{ion} \tag{1.3}$$

The swelling pressure, Π, of a hydrogel matrix is measured by calculating ΔG_{tot} with respect to the moles of water, and is represented as follows (1.4):

$$\Pi = -\frac{\frac{\partial \Delta G_{tot}}{\Delta n_1}}{V_1} = \frac{(\mu_1 - \mu_{1,0})}{V_1} = \Pi_{ion} + \Pi_{mix} + \Pi_{el} \tag{1.4}$$

In equation 1.4, n_1 represents the number of moles of water, V_1 represents the molar volume of water, μ_1 represents the chemical potential of water in the hydrogel at environmental pressure, and $\mu_{1,0}$ represents the chemical potential of water in the solution surrounding the hydrogel.

Flory and Huggins proposed a model for determining the pressure that occurs due to the mixing of polymer chains with solvent molecules. The original equation considers the change in free energy and applies it as a function of the number of moles, n_1, and volume fraction, ψ_1, of solvent 1, the chi parameter, χ, takes into account the energy of interdispersing polymer and solvent molecules, while R and T represent the ideal gas constant and the temperature of the system:

$$\Delta G_m = RT[n_1 ln\varphi_1 + n_2 ln\varphi_2 + n_1\varphi_2\chi_{12}] \tag{1.5}$$

However, the Flory-Huggins equation can be modified to consider the osmotic pressure of mixing of polymer chains with solvent molecules in the following way [43]:

$$\Pi_{mix} = -\frac{RT}{V_1}\left[\ln(1-\varphi) + \left(1-\frac{1}{P}\right)\varphi + \chi\varphi^2\right] \tag{1.6}$$

The variable, P, is the degree of polymerization. Because of the cross-linked structure of hydrogels, P can be considered to be infinite, and the resulting equation is:

$$\Pi_{mix} = -\frac{RT}{V_1}[\ln(1-\varphi) + \varphi + \chi\varphi^2] \tag{1.7}$$

To calculate the elastic pressure that results from hydrogel matrix stretching, rubber elasticity theory is used:

$$\Pi_{el} = -ARTv\sqrt[3]{\varphi} = -G \tag{1.8}$$

In this equation, A is a factor that is dependent upon the functionality of junctions within the polymer matrix, υ is the concentration of polymer elastic chains and G is the shear modulus of the hydrogel matrix, and is defined as the ratio of shear stress to shear strain.

Hydrogels are placed into a solution and swell or deswell depending on the functionality of the side chains within the hydrogel matrix. When a hydrogel has reached equilibrium, the pressure will equal zero.

1.2.4 Kinetics

The mechanism for hydrogel swelling occurs in two steps. First, the stimulus that drives the swelling change must permeate the hydrogel matrix. Second, mass transfer occurs, which results in the swelling or deswelling of the hydrogel. Currently, the hydrogel swelling response is long, taking up to 10 hours for the first order response to occur. Methods must be employed to decrease the response time, and therefore designing hydrogels that could be used in medical applications [40-42].

To explain how hydrogel swelling occurs, Fick's first law of diffusion can be applied. Fick's first law states that the flux moves from a region of high concentration to a region of a lower concentration. The equation given for Fick's first law is:

$$J = -D\frac{\partial c}{\partial x} \tag{1.9}$$

In this equation, J represents the diffusion flux and describes the amount per unit area per unit time ($\frac{mol}{m^2 s}$). The diffusion coefficient, also described as diffusivity, is given by D in units of ($\frac{m^2}{s}$). The concentration, c, and length, x, are represented in units of ($\frac{mol}{m^3}$) and m, respectively. An integration of Fick's first law results in the following equation:

$$J\Delta x = D(c - c_o) \tag{1.10}$$

In this equation, $c\text{-}c_o$ represents the change in concentration across a membrane. One theory [40] defines diffusivity as:

$$D = \frac{(K + \frac{4\mu}{3})}{f} \tag{1.11}$$

where K and μ represent the bulk and shear modulus of the polymer matrix and f represents the friction coefficient between the matrix and the surrounding solution. Finally a model is proposed relating the hydrogel response time to the thickness of the hydrogel sample [40]:

$$R = \frac{x^2}{D} \tag{1.12}$$

This equation states that the swelling response is proportional to the dimensions of hydrogel sample.

1.2.5 Piezoresistive Signal Transduction

The swelling pressure of hydrogels can be characterized in chemomechanical pressure sensor applications with a piezoresistive pressure sensor. Pressure sensors measure the force applied to the sensor in units of force per unit area. While pressure usually refers to the force required to stop expansion, in this case, the pressure is a measurement of the force applied to the pressure sensor by the swelling behavior of the hydrogel. Piezoresistive pressure sensors are also referred to as piezoresistive strain gauges. This type of pressure sensor is made of silicon materials, harnesses the piezoresistive effect, and measures the strain resulting from an applied pressure. Piezoresistive pressure sensors are connected to a Wheatstone bridge to maximize the

signal output and to reduce the error generated in the signal. Pressure sensors provide measurable data in terms of an electrical signal [84-86].

Piezoresistive sensors experience a change in resistance as a result of strain and deformation. Equation 1.13 can be used to calculate the change in resistance:

$$R = \rho \frac{l}{A} \tag{1.13}$$

In this equation, R is the resistance, ρ is the bulk resistivity, l is the length, and A is the cross-sectional area [86]. In piezoresistive sensors, there are two factors that can change the resistance value due to an applied strain. The first is based on the change in the dimensions of both the length and cross-sectional area of the diaphragm. The second is that the resistivity of the diaphragm may change as a function of strain. Therefore, piezoresistors experience a change in resistivity as strain is applied to the diaphragm. Furthermore, the resistivity of a piezoresistive material is dependent upon the mobility of the charge carriers. When the material is subjected to an applied physical strain or deformation, there is a change in the atomic spacing in the semiconductor lattice, which results in a change in the bulk resistivity of the material [84].

Piezoresistive strain gauges include piezoresistive sensing diaphragms that are bonded on the perimeter with rods that are subjected to external loading forces. The change in resistance is measured with a Wheatstone bridge, which consists of four resistors that have been connected in a loop, as shown in Figure 1.4. In a Wheatstone bridge, the input voltage is applied across two junctions that are each connected to a resistor. As the signal passes through the circuit, there is a voltage drop across the other two resistors. The output voltage is a result of the drop in resistance across the circuit.

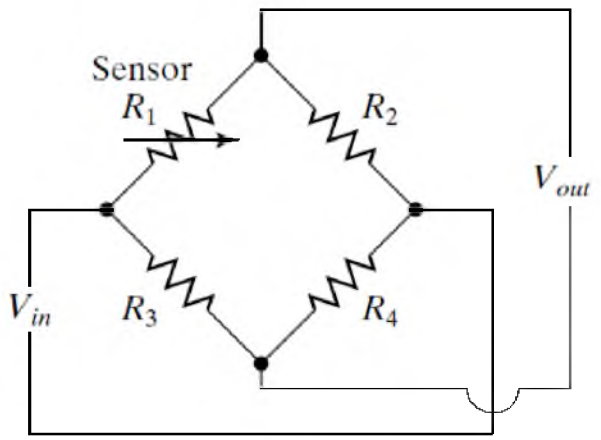


Figure 1.4. A schematic circuit diagram of a Wheatstone bridge [85].

Equation 1.14 demonstrates how the output voltage is related to the drop in resistance across the Wheatstone bridge [85].

$$V_{out} = \left(\frac{R_2}{R_1 + R_2} - \frac{R_4}{R_3 + R_4}\right) V_{in} \qquad 1.14$$

Chemical sensors consist of a signal recognition component and a signal transduction component. The hydrogels designed in this project represent the signal recognition component, while the piezoresistive sensing diaphragm provides the signal transduction method. In the case of the chemomechanical, piezoresistive pressure sensors, the resistors are located in the center in the regions of maximum tensile stress when a uniform pressure is applied to the sensing diaphragm [85]. This provides a method for being able to detect the change in swelling pressure of the hydrogel-based on the concentration of the media solution.

1.3 Current Research

The swelling and deswelling behavior can be measured through hydrogel characterization techniques or through signal transduction methods. Signal transduction mechanisms must convert nonelectrical changes of the material into a measurable electrical signal. Current methods include optical, conductometric, amperometric, microcantilevers and bending plate transducers [46-74].

1.3.1 Summary of the pH-Response Characterization Methods

Hydrogel characterization methods have been used by some research groups to measure the swelling behavior in addition to characterizing the cross-link density and water absorption of the hydrogel matrix.

1.3.1.1 Weighing Methods

The swelling response of pH-responsive hydrogels has been characterized by some research groups with weighing methods [44-46]. Hydrogel samples are placed into a tea bag and weighed to determine the difference in weight, and consequently volume, of the hydrogel both before and after swelling. Hydrogels are synthesized and placed into a tea bag [44]. All excess moisture is removed from the sample, and is weighed to determine the initial weight. The tea bag is then placed into a different media solution. In some projects, the weight is measured at specific time intervals, to ensure the swelling behavior is characterized [46]. Other research projects have simply used this method to measure the difference between the volume between both the swollen and unswollen states of the hydrogel [46].

1.3.1.2 Other Methods

One research group utilized Fourier Transform Infrared Spectroscopy, thermogravimetric analysis, and rheomechanical spectrometry methods in addition to the weighing method not only to characterize the swelling behavior of the hydrogel, but also to analyze the cross-linking, water absorption, and the modulus of the cross-links within the hydrogel matrix [47].

Another research group used microbeads that had been incorporated into the hydrogel before polymerization to measure the mechanical properties [42]. Hydrogel samples were injection molded into a dumbbell shape. The microbeads became a built in ruler, and the researchers used an optical camera with a high resolution to image the hydrogel at specific time intervals during the experiment to measure the change in distance between the microbeads in the hydrogel sample.

1.3.1.3 Optical Transducers

Optical methods measure the changes in optical properties of the hydrogels. Some compositions of hydrogels change their optical transmission after swelling, and may turn either transparent or opaque [26,32,48,73]. Researchers have also coated the surfaces of hydrogels with gold nanoparticles or other particles to measure the change in the distance of the hydrogels due to swelling. The changes in refractive index and even reflection of the hydrogels have been measured as an indicator of the degree of swelling [50]. In addition, some researchers have labeled hydrogels with fluorophores as a way to measure the degree of swelling based on fluorescence intensity [46,47].

1.3.1.4 Reflective Diaphragms

For reflective diaphragm methods, a hydrogel is coupled with a sensing platform and a reflective plate. As the hydrogel swells, the plate is moved, which causes a displacement of the reflective surface. As the light is reflected back to the sensing platform through an optical fiber, the sensing platform measures a change in the intensity of the light [48].

1.3.1.5 Fiber BRAGG Grating Sensors

Hydrogels are used to coat a Fiber BRAGG Grating sensor. The BRAGG wavelength is shifted due to hydrogel swelling. The source light travels through a fiber and the BRAGG wavelength is reflected by the grating on the Fiber BRAGG Grating sensor. This method was developed by a group that observed that the stress induced by hydrogels is weak and most of the stress expended is utilized in straining the cladding on the fiber. This method was developed to measure the full degree of swelling of the hydrogel [50].

Another group also utilized a Fiber BRAGG Grating sensor in combination with optical time domain reflectometry. This group concluded that the detection of hydrogel swelling is a function of linear position along the fiber length and that with this method, the swelling exerts enough force to generate a highly sensitive signal, on the order of nano-pH levels [13-15].

1.3.1.6 Microgravimetric Transducers

For microgravimetric signal transduction, a mass sensitive quartz crystal microbalance was used. A hydrogel was coated on one side with resonators of quartz. As the hydrogel swells and deswells, the surface load changes, which changes the surface resonance frequency. Surface Plasmon Resonance was used to measure optical thickness. This method provides the ability to detect very small changes in the swelling behavior [57].

Another research group utilized the resonance frequency to measure hydrogel swelling. This group designed a microelectricalmechanical system to measure the change in distance between two plates, one flexible the other rigid. The goal of this group is to design microsensors that can be implanted into the body. The device included an integrated wireless sensor and a diaphragm that could be deflected. The signal transducer incorporated a resonant LC, inductor/capacitor, circuit and the analyte concentration was determined from the resonance frequency measurements [51].

1.3.1.7 Fluorescence

The swelling behavior of hydrogels can also be characterized with fluorescence. In one research project, for example, the pH-sensitive hydrogel was loaded with RAST, an orange-red fluorescent marker. The sensor consisted of microchannels that were prepared with the hydrogel pregel solution, containing pH sensitive fluorophores. The polymerized hydrogel was imaged with a monochrome charge-coupled device camera and filters were used to capture the fluorescence induced by excitation in transmission

mode. The pH response range was from 6.0 to 8.0, but photobleaching occurred as a result of the imaging, which decreased the sensor stability [16].

Another project created a pH sensor that uses SNARF and carboxylic acid to detect changes in pH without introducing a dye that would interact with the cells of the bioreactor. The pH sensitive dye had been characterized over a range of 5.5 to 9.0 and is called phenol red. The dye was tested in both cell culture media and PBS. A hydrogel with dimensions of 500 μm in diameter and 1000 μm in thickness was loaded with the dye by entrapping the dye into the hydrogel matrix. The hydrogel was polymerized with UV exposure. The hydrogel was tested and no leaching occurred. In addition, the pH response was linear with no change in response between the PBS solution and the cell culture media. The hydrogel composition included a backbone of polyethylene glycol (PEG), which is biocompatible and nontoxic. In addition, this hydrogel formulation has proven a resistance to protein adhesion. The goal of the research group is to integrate the hydrogel into bioreactors and cell-containing microanalytical devices. The response time of this hydrogel was 10 minutes. The researchers concluded that the hydrogel response time was slow because the hydrogel acts as a buffer through its interaction with the carboxyl groups, which causes a slower binding time with hydrogen ions in solution [46].

A third project that utilizes fluorescence also employed the use of PEG hydrogels by designing microchannels filled with hydrogel pregel solution containing pH sensitive fluorophores. The microarray generated components that measured 100 μm. The hydrogel response was imaged with a CCD camera and filters that were used to capture the fluorescence induced by excitation in transmission mode. A pH range of 6-8 was

detected; however, photobleaching occurred, therefore affecting the pH sensitivity. In addition, the sensor had a decreased stability due to experimental conditions [48].

1.3.1.8 Other Optical Methods

Another optical method is measuring the holographic diffraction wavelength. Holographic diffraction gratings are illuminated by white light, and result in sensitive wavelength filters. As the hydrogel swells, it generates interference between the incident light and reflected beam. This process has a very high sensitivity, but is also complicated with many components [52].

1.3.2 Mechanical Transducers

Mechanical signal transduction is used by some groups to harness the mechanical work of the hydrogel as it swells. Two classes of mechanical signal transduction include microcantilevers and bending plate transducers.

1.3.2.1 Microcantilevers

The measurement obtained from microcantilevers is based on the same functionality as atomic force microscopy, which takes advantage of biomolecular interactions on the surface of the cantilever. A hydrogel is placed on the surface of the microcantilevers. As the target analyte or biomolecule interacts with the hydrogel, the hydrogel swelling behavior is converted into nanomechanical motion. This motion is measured in combination with either optical detection via lasers or with piezoresistive bending plates [36].

1.3.2.2 Bending Plate Transducers

Bending plate transducers harness the mechanical work of the hydrogel as it swells, and utilize a piezoresistive sensing diaphragm for detecting the change in mechanical pressure applied to the sensor. A piezoresistive diaphragm utilizes a silicon membrane, which has a high piezoresistive effect. The piezoresistive effect describes the ability of a material to increase its resistance as a strain is applied. As the diaphragm bends, the interatomic spacing of the material increases, which changes the ability of the material to raise electrons to the conduction band [84]. The hydrogel sample is confined and placed on a piezoresistive membrane, and the swelling properties of the hydrogels are used to generate a voltage that can be collected and analyzed to determine the degree of swelling and the response time of the swelling hydrogel (see Figure 1.5) [54-74].

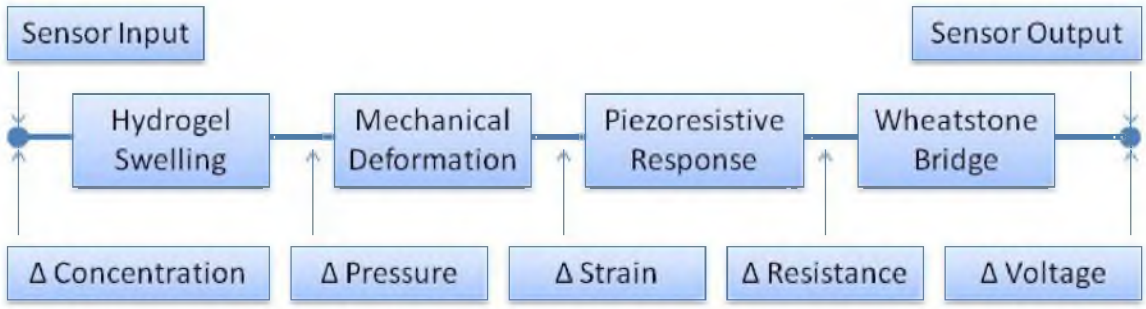


Figure 1.5. Piezoresistive signal transduction mechanism: the hydrogel swells as a result of a change in analyte concentration, the piezoresistive diaphragm is deformed, resulting in a piezoresistive response. A Wheatstone bridge is used to change the mechanical signal to a voltage output. The change in voltage is analyzed to determine the response time and magnitude.

In one research project, a hydrogel sample was integrated into a piezoresistive sensor with spin coating. Spin coating was used to control the thickness of the hydrogel. The hydrogel was incorporated into the sensor, and a small void was left between the hydrogel surface and the piezoresistive membrane. The void created a method for measuring the completely dried state of the hydrogel. The sensor utilized microfluidic channels to control the concentration of the analyte in solution. The composition of the hydrogel used generated an unstable signal that was the result of the slow continuous change in the electrical potential at the hydrogel-solution interface. This caused signal drift and made it difficult to characterize and calibrate the sensor. In addition, this group has also noted that phase transitions occur as the hydrogel samples reach the upper and lower critical solution temperatures. Furthermore, they conclude that hydrogel conditioning is necessary for high signal reproducibility and that the sensitivity is dependent upon both the composition of the hydrogel and the degree of cross-linking [54-60].

Another research group synthesized hydrogel monoliths with 400 μm thickness. They tested the monoliths under various media conditions to determine the swelling response of both the free swelling and confined samples. Hydrogel samples were confined on all sides except for one side, which interacted with the media solution through a porous mesh membrane. The group noted that the degree of swelling of unconfined hydrogels is larger than that of confined hydrogels; however, confined hydrogels can still be accurately measured when placed in a confined space with a porous rigid membrane [61-67]. This group further explored the temperature and ionic strength responses. In all experiments where the hydrogel was confined, the hydrogel sample was

placed directly on the piezoresistive sensing diaphragm, and a cap was placed on the pressure sensor and hydrogel sample. The cap was tightened to create a loading pressure, so that the full hydrogel response could be measured. The hydrogel thickness and composition were not optimized in these experiments, which resulted in excessively long response times. In addition, the hydrogel samples demonstrated a high sensitivity to small changes in environmental conditions.

A third group synthesized pH-responsive hydrogels with a HEMA backbone and DMA sensing groups. Their objective was to use the hydrogels with a pressure sensor to calculate the partial pressure of CO_2 in a solution. This was done by loading the hydrogel sample into the pressure sensor, adding a well of dissolved CO_2 and utilizing a Severinghaus membrane, which is permeable to CO_2. The concept is that CO_2 reacts with the bicarbonate solution, which decreases the pH, and results in a swelling response by the hydrogel. This group also confined the hydrogel inside the pressure sensor by creating a fixed volume. A pressure was generated because the swelling behavior was measured with the piezoresistive diaphragm. This group experimented with both microspheres and thin layers of hydrogel. The microspheres presented challenges in that the spheres would move during hydrogel swelling, which resulted in noise and hysteresis. Furthermore, the microspheres were difficult to handle, hard to dose, hard to confine, and required the use of a smaller mesh size, which decreased the rate of diffusion through the membrane. Because of these effects, the group changed their design and synthesized hydrogels of 750 μm in diameter and 50 μm in thickness. The thin layered hydrogels generated a more stable signal, were easier to handle, and allowed the use of a membrane with a larger pore size, which resulted in faster diffusion. The response time was 1000

seconds (see Table 1.1). The group hypothesized that a decrease in hydrogel thickness should decrease the response time, but would also decrease the response magnitude. Therefore, they propose that there must be a balance between the response time and magnitude. This group has not published any additional project data [69-74].

1.3.3 pH-responsive Hydrogel Results

There are several research groups who have designed methods for signal transduction of the hydrogel swelling behavior. There are also many different hydrogel compositions that have been designed to have a response to the change in analyte concentration. The results of some of the analyte-responsive hydrogel used by other research groups are listed in Table 1.1.

The data presented in Table 1.1 compare the hydrogel composition, thickness, response time, and signal transduction across several research projects. Not all of the research projects provided results for the composition, thickness, or response time in their projects because those parameters were not the main focus of their research. They are provided here to illustrate the inconsistency of these parameters among research projects.

1.3.4 Challenges of Current Hydrogel Research Projects

Currently there are no fast, noninvasive methods for quickly measuring the systemic pH levels in clinical settings [5]. There is a clinical need not only for continuous pH monitoring, but also a rapid method to determine if a patient is in shock, so the shock can be treated first without further jeopardizing the patient by treating serious injuries first [1-3].

Table 1.1. A comparison of analyte-responsive hydrogel results from reference projects with the composition, hydrogel thickness, response time, and the signal transduction method used in the project

Signal Transduction Method	Hydrogel Backbone/Sensing Group	Hydrogel Thickness	Response Time	Reference
Piezoresistive	Polyvinyl Alcohol/Poly acrylic Acid	50 μm	50 sec	55
Quartz Microbalance	Polyvinyl Alcohol/Poly acrylic Acid	1.1 μm	50 sec	57
Piezoresistive	Polyvinyl Alcohol/Poly acrylic Acid	6 μm	1.2 sec	54
Piezoresistive	Polyvinyl Pyridine	4 μm	70 sec	56
Piezoresistive	Phenyl Boronic Acid	400 μm		61
Optical	Polyvinyl Alcohol	37.5 μm	40 min	50
Optical	Polyethylene Oxide	40 μm	45 min	12
Various Measurement Techniques	Hydroxyethyl Methacrylate/Polyacrylic Acid	150 μm 200 μm 300 μm	41 min 133 min 266 min	42
Optical	Acrylamide/Bis Acrylamide	125 μm	200 min	13
Optical	Polyacrylic Acid		40 min	15
Piezoresistive	Hydroxypropyl Methacrylate/ Dimethylaminoethyl Methacrylate	400 μm	2.25 hours	62-67
Capacitive	Hydroxyethyl Methacrylate	60 μm		51
Piezoresistive	Polyvinyl Alcohol/Poly acrylic Acid	40 μm	4700 sec	58
Conduction	Hydroxypropyl Methacrylate/ Dimethylaminoethyl Methacrylate	750 μm 8 μm		53
Microcantilever	Polyvinyl Pyridine			36
Holographic Optical	Hydroxyethyl Methacrylate/Polyacrylic Acid		250 sec	52
Weighing Methods	poly[*N*-vinyl-2-pyrrolidone-polyethylene glycol diacrylate]		3 hours	44
Piezoresistive	Hydroxypropyl Methacrylate/ Dimethylaminoethyl Methacrylate	50 μm	15 min	69-71
Weighing Methods	Acrylamide/Bis Acrylamide		250 min	45
Mixed Methods			6 min	47
Optical/ Fluorescence	Polyethylene Glycol with RAST	1000 μm	15 min	46
Weighing Methods			100 min	78

In addition to the need for pH monitoring of physiological conditions, researchers who work with bioreactors need a reliable method for monitoring the pH within bioreactor systems. Each of the current methods has flaws, whether from mechanical instability or reactivity with the bioreactor environment [4-14]. Therefore, it is important to create a device that will allow researchers to monitor the pH of a system without changing the composition of the cell culture media and without interfering with the function of the bioreactor [7].

With regard to hydrogel-based sensors, research projects designed to exploit the swelling response of hydrogels have all noted that the response time of the hydrogel swelling response is longer than would be desirable in a commercial sensor for any application [11-74]. Therefore, researchers have investigated various methods for capturing and measuring the stimuli response of hydrogels [54-74]. Some of these methods include the synthesis of nanocomposite hydrogels [78], microbeads [33], and the design of amphiphilic molecules that form supramolecular hydrogels [80-83]. These have all been designed to detect either analytes in solution or biological compounds. Many of the signal transduction methods currently used are complicated and require the use of many instruments to measure the change in swelling pressure of the hydrogel.

One of the foremost challenges of utilizing hydrogel sensors for continuous monitoring is that failures in sensor performance lie in the irregularity of analytical performance [22-39]. In order to meet the demands of continuous monitoring, pH sensors must be reliable [22-39]. They must also be self-contained devices that provide quantifiable information in direct contact with the media solution; function reliably for

hours without physical, chemical, or signal degradation; and must have a fast response and high sensitivity to small changes in the local environment [22-39].

1.4 Summary of Literature Review for Bioreactor pH Sensors

Monitoring the pH of bioreactor solutions is the key to the success of bioreactor operations [6]. The majority of the current pH monitoring methods for bioreactor applications requires removing samples from the bioreactor for the pH test [4-10]. While the pH must be closely monitored to avoid fouling, each time the media solution is sampled, it increases the likelihood of contamination of the media solution. One research group observed fouling in their research project [7]. They demonstrated that pH monitoring through sampling reveals the conversion of nutrient ions to ammonia via nitrification in the aerobic phase. Complete denitrification occurs in the anoxic phase resulting in nutrient removal. In addition, phosphate uptake occurred during the cyclic phases of the bioreactor.

Current pH monitoring methods include ionic sensitive field effect transistors (ISFET), steam sterilizable glass pH electrodes, and optical methods using fluorescence [4-15]. ISFET devices, for example, may have an effect on cellular physiology. All monitoring devices must be inert [4], and because of this, ISFET devices may not be optimal for bioreactor applications. Glass electrodes have been successful, and are currently used, but they have a low mechanical stability [4].

Optical pH monitoring of bioreactor systems may be more promising than ISFET and glass electrode monitoring. These methods utilize nontoxic, visual pH indicator dyes, including phenol red (590 nm). The dye is used in combination with a

spectrophotometric plate reader and the pH is calculated from the absorption. This specific method is highly accurate, but requires sampling from the bioreactor [9].

One group developed a fluorescent pH monitoring system for online (continuous) pH detection [10]. This group developed an optical sensor for the detection of phenol red, which they claim is a normal component of cell culture medium. The new device is noninvasive and has been designed to fit the shape of the bioreactor chamber. They developed a microfluidic chip with an oval shaped detection chamber to eliminate the flow dead zones and reduce the response time. Optical fibers are connected to a LED as a light source and another fiber is coupled with ST silicon PIN photodiode as a photon receptor. The optical signal is converted to voltage by way of the photodiode and a custom-built signal amplifier. The media solution is channeled through the sensor via a polydimethyl siloxane microfluidic channel system, with an optimal thickness of 200 μm. The results obtained from the project were first from computational methods and validated with experimental results.

Another group created a device that uses fiber optic cables, a Shimadzu spectrophotometer, a light source, a quartz optical flow cell, and a photodiode with a signal amplifier [6]. The pH was measured by comparing the ratio of green to red in the cell culture media solution. This group reported that there was no biofilm build up on their device. In addition, their device performed well with no need for recalibration after multiple cycles.

Optical monitoring of the pH of bioreactor cell cultures has proven promising, but the dyes used have a narrow range with a range near the pKa of the dye used [4]. In addition, they depend upon the presence of fluorescent dyes, including phenol red, in the

cell culture media. The presence of dyes has not demonstrated adverse effects, but it represents the need to include additional compounds that could react over time with the contents of bioreactor systems.

Current pH monitoring methods for bioreactor applications have not met the needs of the researchers who use them. Monitoring devices must be inert [4], and because of this, optical devices may not be optimal for bioreactor applications. In addition, glass electrodes, which are currently used, have a low mechanical stability [4], which requires recalibration during bioreactor processes. All other developed methods for monitoring the pH of cell culture media in bioreactors require sampling, which is not ideal and should be used for limited applications. Methods for continuous monitoring the pH of physiological conditions are virtually nonexistent and currently do not account for user error.

1.4.1 Potential Advantages of Chemomechanical pH Sensors

Chemomechanical sensors that can monitor the pH of media conditions would meet the needs of both the bioreactor and biomedical industries. Stimuli-responsive hydrogel-based sensors have the potential to provide continuous monitoring applications in real-time and have the potential for a higher stability than methods currently used. In addition, hydrogel sensors have a longer shelf life and then can be sterilized with gamma sterilization, unlike enzymes and antibodies that are needed for bioreactor use.

As outlined above, there are many research groups what are focusing on the use of optical methods to characterize the swelling response of pH-sensitive hydrogels.

These methods demonstrate a high sensitivity to small changes in pH, but are also quite complicated. Sensors that measure the change in pH of a biological system should be self-contained, and should not require the user to sample the media solution. Because of the complex methods employed by researchers for measuring the change in swelling pressure, chemomechanical sensors may prove to be a more viable option because they can be manufactured quickly and at low cost. The entire sensing platform can be contained within the device. The pH-sensitive hydrogel material can be placed in direct contact with the media solution, and the signal can be transduced immediately and in situ. Furthermore, chemomechanical sensors can provide continuous measurements.

The methods and results presented in this project have been designed to fill the need for reliable continuous pH monitoring devices for bioreactor systems. The needs outlined not only include the reliability of the devices, but also self-contained devices in direct contact with the media solution that maintain the sterility of the environment and neither react with the environment nor catalyze a reaction within the bioreactor system. Furthermore, this project will help fill the need of reliable function for an extended period of time without a degradation of the material or signal, while maintaining a fast response and a high sensitivity.

1.5 Thesis Overview

1.5.1 Goals

As shown by current research projects, there are few reliable methods of monitoring the pH in bioreactors. The variation in pH within a bioreactor system could

result in the fouling of the contents of a bioreactor; therefore, it is imperative that the pH measuring methods are reliable to ensure proper control of the bioreactor system.

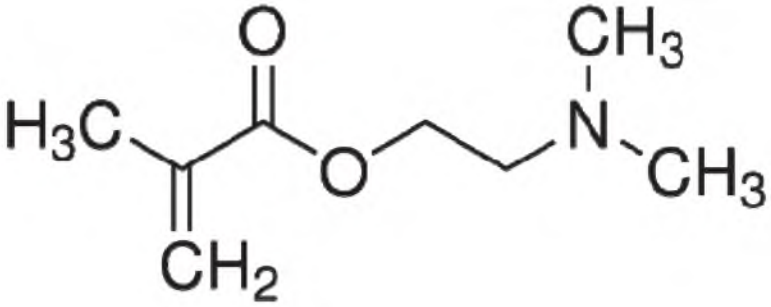
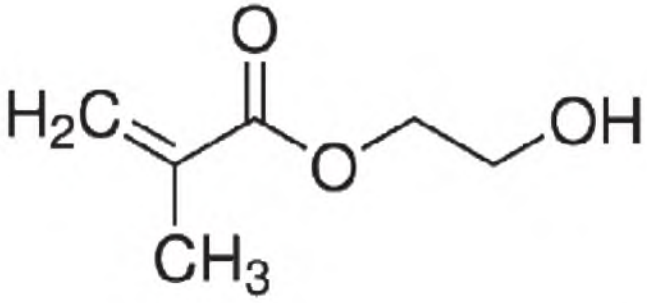
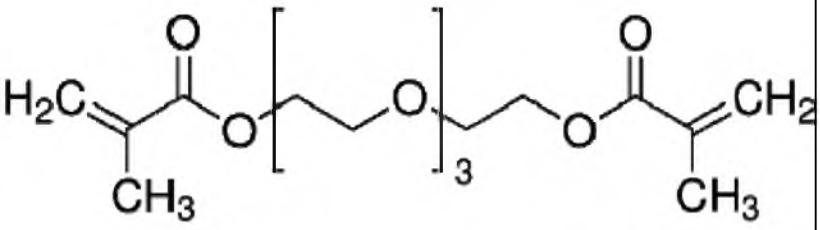
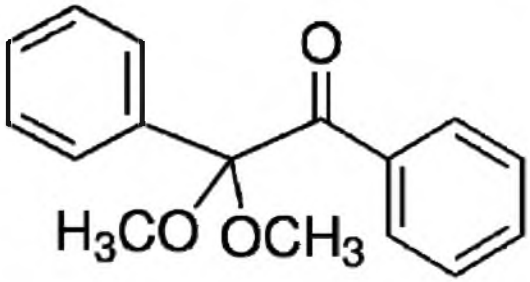
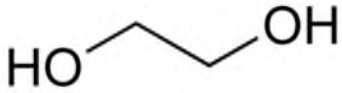
This thesis project focuses on the optimization of the hydrogel swelling response time. In addition, the methods of signal transduction will be assessed to determine an efficient and reliable method for continuous monitoring of bioreactors and other biological systems. In addition, there is no mention in published data of the ability to store hydrogel-based sensors for an extended period of time or of their continued response across continuous cycles of testing; therefore, the longevity of the hydrogel swelling response will be tested to determine a minimum shelf life.

1.5.2 Response Characterization of Hydrogels Designed for Use in Microfabricated Sensor Arrays

A preliminary study of a hydrogel composition will be presented to determine the response of the hydrogel to small changes in pH as well as changes in concentration of other solutions. The monomers presented in Table 1.2 will be used.

The solutions tested in these experiments will contain analytes to which hydrogels have been proven to respond [25-35]. The swelling behavior of the hydrogels used in this project will generally utilize a piezoresistive sensor. The hydrogel will be placed on the sensing diaphragm and confined on most sides. Confining the hydrogel samples has demonstrated that confined hydrogels will swell in the y direction as opposed to the x, y, and z directions (see Figure 1.6) [32].

Table 1.2. Materials and Chemical Structures

Chemical Name	Chemical Structure
2-(Dimethylamino)ethyl methacrylate	
2-Hydroxyethyl methacrylate	
Tetraethylene glycol dimethacrylate	
2,2-Dimethoxy-2-phenylacetophenone	
Ethylene glycol	

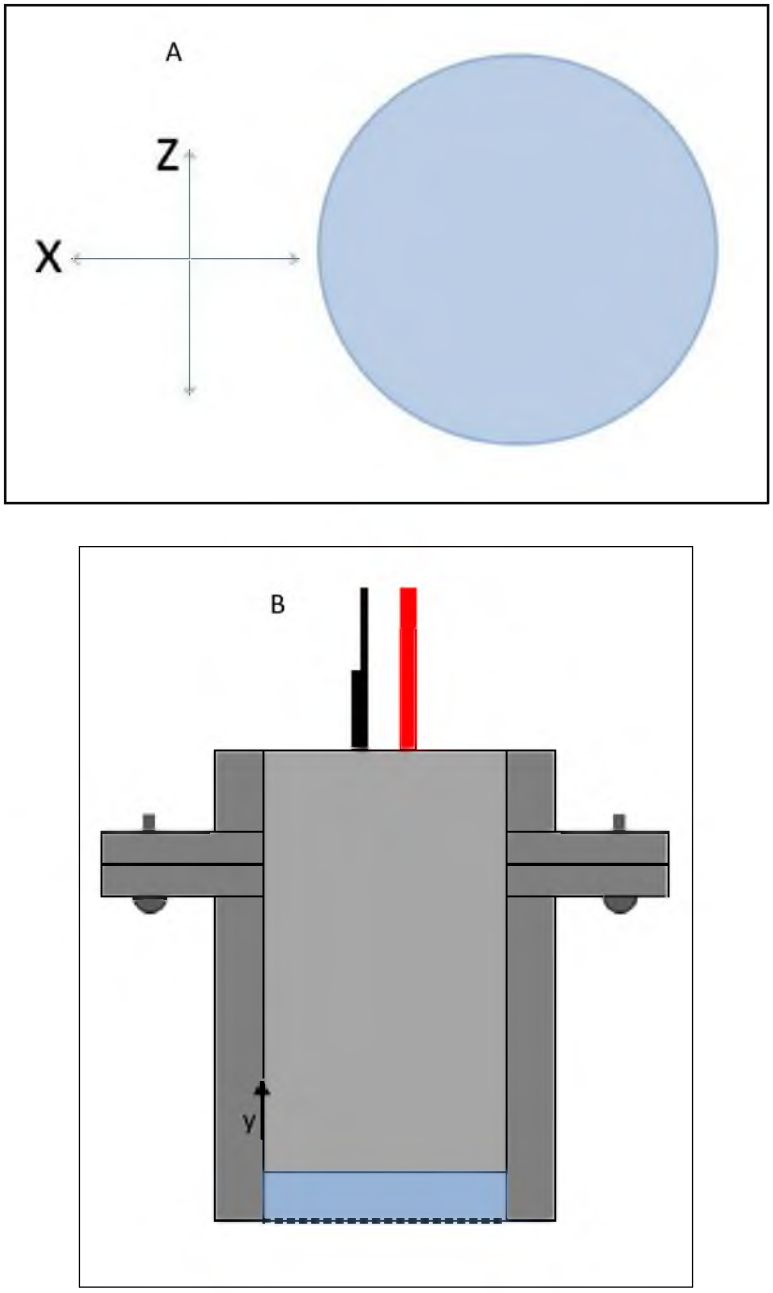


Figure 1.6. Image A illustrates the unconfined hydrogel response in the x and y directions, while Image B illustrates the swelling of a confined hydrogel in the y direction only.

The hydrogel samples will be covered with a stainless steel cap that includes a steel mesh, which will allow the hydrogel to interact with the surrounding environment. The gel will be loaded into the sensor and placed into varying media solutions. As the gel swells, it will apply a mechanical force to the piezoresistive membrane and a voltage will be generated. The voltage will be used to determine the magnitude of the swelling in each solution and will also be used to determine the response time of the hydrogel sample to various solutions. While this method will be the most used in this project, other methods of determining the hydrogel response will also be utilized, and will be discussed in their respective chapters.

1.5.3 Hydrogel Response Optimization and Chemomechanical pH Sensor Response Time and its Dependence on Hydrogel Thickness

Hydrogels in past and current research projects have not demonstrated an ideal response time. Therefore, hydrogels will be altered both chemically and mechanically with the goal of decreasing time of the swelling response. The hydrogel composition will be modified with respect to the amount of backbone molecules, sensing groups, solvent, and cross-linking molecules to determine the effect of the modification as well as tradeoffs that may result in the altering the hydrogel composition. Hydrogel monoliths will be synthesized with a thickness of 400 μm to establish a baseline for the response time and magnitude of varying compositions. However, the thickness will be altered to determine the effect of thickness on analyte diffusion through the hydrogel matrix.

Hydrogels will also be mechanically altered by perforating the samples to determine how diffusion changes as a result of mechanical alteration.

1.5.4 Shelf Life and Longevity of Stimuli Swelling Response

Hydrogels of an initial composition will be synthesized and tested at various time intervals to determine whether hydrogel-based sensors may be stored and used over a period of time, or if they will need to by produced and used immediately. Furthermore, hydrogels will be synthesized, dried, and shipped overseas and tested to determine how the hydrogel responds to vibrations and other shipping conditions.

In addition to testing the shelf-life of hydrogels, there is no published data regarding the continuous testing of hydrogels to determine if the response magnitude decreases over time. Hydrogels will be tested for multiple cycles to determine how the hydrogel responds to repeated testing.

1.5.5 Piezoresistive Sensor Design: The "Boss" Sensor and a Chemomechanical Sensor Using 3D Printing Technology

The swelling response will be characterized on one sensor design for initial results to determine which hydrogel composition is ideal for hydrogel-based sensor applications. Hydrogel samples will then be tested with another piezoresistive sensor assembly, called the Boss sensor because of a protrusion on the sensing surface that is mechanically attached and limits the thickness of hydrogels used. The objective is to utilize an off the

shelf, more economical sensor diaphragm that will provide the same response as the less economical, lab-grade sensor used for the preliminary characterization of the hydrogels. The silicon sensor will be mechanically attached to a "boss" device, and hydrogel samples will be polymerized in situ. Results will be compared to those obtained from the lab-grade sensor to illustrate the effectiveness of the sensor design.

1.6 Novelty

As outlined above, several groups have focused their research on pH-responsive hydrogels. While these groups have been successful in characterizing the pH-response, their methods have all varied. In addition, current signal transduction methods of pH-responsive hydrogels have not been designed to meet the needs of the industry, which include self-contained sensors that do not require sampling of the cell culture media, continuous monitoring, high stability without the need for frequent recalibration, and non-reactive with the contents of either the cell culture media or physiological conditions. Furthermore, if chemomechanical hydrogel sensors are to be used in a clinical or industrial setting, the response time must be fast, and the signal must be distinguishable from the noise. The research projects outlined above have characterized the pH response, but have noted that the composition has yet to be optimized. The experimental data presented in this thesis help meet the needs of both the clinical and industrial applications. The long-term hydrogel response was characterized to demonstrate its ability to respond over time, the full stimuli response was characterized, the composition and thickness were optimized, and the hydrogel was integrated into various chemomechanical sensor designs, including a mechanical boss sensor and sensor

integrating 3D printed parts. The results presented in the chapters could be used to create a chemomechanical sensor that meets all of the outlined requirements.

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CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

Hydrogels have demonstrated their ability to respond to environmental stimuli, including changes in ion concentration, pH, ionic strength, glucose concentration, and other analytes. The hydrogel response occurs as a change in swelling pressure. pH-responsive hydrogels swell as the pH decreases and deswell as the pH increases. The swelling mechanism occurs as a result of the protonation of the nitrogen groups on the side chain of dimethylaminoethyl methacrylate [1-33].

The swelling pressure in this project was measured by weighing methods as well as by signal transduction, where the nonelectrical changes in the hydrogel are converted to a measurable electrical signal. The signal transduction method used was a bending plate piezoresistive pressure sensor. Hydrogel samples were loaded into the pressure sensor, and a plot of pressure versus time is created [1-33].

Due to the responsive nature of hydrogels, hydrogel-based sensors have been proposed as a method of measuring changes in aqueous environments found both in the human body and in bioreactor applications [34].

Hydrogels used in this experiment were synthesized by free radical polymerization with UV curing methods. Samples were removed from the hydrogel monolith and tested with a piezoresistive pressure sensor.

2.2 Materials

The following monomers were used as received from Sigma Aldrich: 2-hydroxyethyl methacrylate (HEMA), dimethylaminoethyl methacrylate (DMA), and tetraethylene glycol dimethacrylate. In addition, 2,2-dimethoxy-2-phenylacetophenone (DMPAP), a photoinitiator, and ethylene glycol (EG), a solvent for the pregel solution, were also obtained from Sigma Aldrich and used as received. Dulbecco's phosphate buffered saline was also obtained from Sigma Aldrich and mixed at 9.6 g/L in deionized water (see Table 2.1).

Table 2.1. Chemicals used for polymerization and testing.

Name	Company	Catalog Number	Comments
2-Hydroxypropyl methacrylate	Sigma Aldrich	868-77-9	Stored at 4 ^{o}C
Dimethylaminoethyl methacrylate	Sigma Aldrich	2867-47-2	Stored at 4 ^{o}C
Tetraethylene glycol dimethacrylate	Sigma Aldrich	109-17-1	Stored at 4 ^{o}C
2,2-Dimethoxy-2-phenylacetophenone	Sigma Aldrich	24650-42-8	
Ethylene glycol	Sigma Aldrich	107-21-1	
Dulbecco's Phosphate Buffered Saline	Sigma Aldrich	231-791-2	
Hydrochloric Acid	Fluka	7647-01-0	Diluted to 0.1 M

2.3 Hydrogel Synthesis

2.3.1 Monolith Synthesis

The hydrogel samples were prepared with two methods for this project. Monoliths were prepared in a synthesis module and samples were also prepared for a microsensor in situ in a glove box. For hydrogel monolith preparation, the monomers were removed from storage prior to synthesis to prevent monomers from reacting with the water in the air. The synthesis module was assembled (see Figure 2.1), which consists of two glass plates and a polymer spacer. The spacer used in this experiment is made of Teflon and is 400 μm in thickness. The resulting hydrogels are hydrophobic around pH 7.0 and are easily separated from hydrophilic glass plates. Furthermore, UV light will not penetrate transparent polymer materials as easily as glass. Therefore, glass is an ideal material for the synthesis module of pH-sensitive hydrogels. The synthesis module is clamped together with mechanical clips.

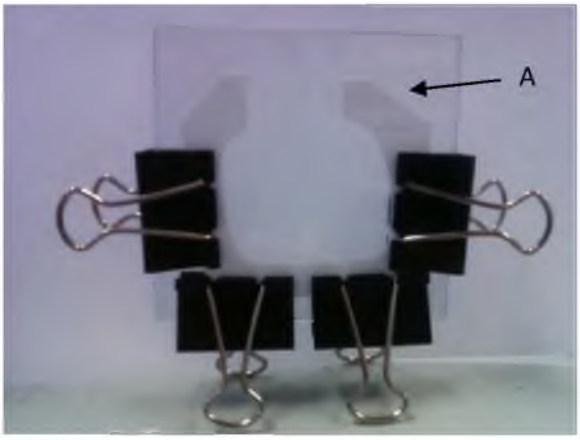

Figure 2.1. This is the synthesis module used to synthesize the hydrogel monoliths. A is the 400 μm Teflon spacer used to control the thickness of the hydrogels.

The synthesis module was then placed under a ventilation hood and purged with argon gas for 5 minutes. This was done by attaching a PVC hose to an argon tank and using a small hypodermic needle attachment. The needle attachment was placed into the synthesis module.

The pregel solution, which consists of monomers and photo initiator in solution, was then assembled. The solution was prepared in a 1000 μL test tube by adding the monomers in a specific order. First 2,2-dimethoxy-2-phenylacetophenone, a photo initiator, was added to the test tube. Then the following monomers were added: 2-hydroxypropyl methacrylate, dimethylaminoethyl methacrylate, and tetraethylene glycol dimethacrylate. The pregel solution was then stirred vigorously with a vortex machine for 5 minutes, until the crystals of 2,2-dimethoxy-2-phenylacetophenone were dissolved and no longer visible. A certain volume of of ethylene glycol was added to the pregel solution and the solution was stirred again for 2 minutes. The photo initiator had a low solubility in ethylene glycol, so it was important to dissolve the photo initiator in the monomers prior to the addition of ethylene glycol. The pregel solution was also purged with argon gas for 5 minutes by placing the needle attachment into the solution and allowing small bubbles of argon gas to form from the tip of the needle at the base of the test tube.

A synthesis station was created by placing a white sheet of paper in the ventilation hood, with a structure on either side that measures ½ inch. This kept the UV lamp from touching the synthesis module. A specific volume of pregel solution was injected into the synthesis module with a micropipette. This was done by placing the tip of the

micropipette at the top of the synthesis module between the two glass plates and slowly releasing the pregel solution into the module.

The synthesis module was placed on the white sheet of paper, and the UV lamp (365 nm) was placed over the synthesis module. The UV lamp was turned on, and polymerization took approximately 90 seconds. The hydrogel was removed the hydrogel monolith from the synthesis module by first removing the mechanical clips and then forcing deionized water into the synthesis module. A metal spatula was used to force the synthesis module open very slowly and carefully. The hydrogel monolith would adhere to one side of the module. The hydrogel was hydrated with deionized water until it was uniformly white. Deionized water was used in combination with a metal spatula to lift the hydrogel monolith from the glass surface. The monoliths were stored in a 100 mL storage bottle with 165 mM PBS solution.

2.3.2 In Situ Synthesis

2.3.2.1 Surface Preparation

The silicon surface of the micropressure sensor was hydrophilic; therefore, surface preparation with 5% APTES in ethanol was used to increase adhesion of the hydrogel pregel solution to the silicon surface. The silicon wafer was treated with 5% APTES by dipping the wafer in the solution three to five times and then immediately removing the residue in deionized water. The solvent was removed from the surface by low pressure argon blowing. A small amount of hydrogel pregel solution was synthesized with UV exposure on the surface of both treated and untreated silicon wafers, and adhesion was increased on the treated surface. After testing the experimental

procedure on a silicon wafer, and determining that the adhesion was increased on the silicon surface, the surface of the pressure sensor was treated with APTES.

2.3.2.2 Synthesis

The pressure sensor was preassembled, and the hydrogel was injected through the mesh membrane. The hydrogel was synthesized in a glove box in an inert environment. The sensor was placed under a microscope and 1.5 μL of the pregel solution was placed on the surface of the mesh membrane. The pregel solution was observed until it had passed through the mesh membrane. UV light, 365 nm, was immediately applied to the pregel solution until polymerization was complete (see Figure 2.2). The hydrogel was not hydrated until testing began.

2.4 Hydrogel Conditioning

The hydrogel monoliths are allowed to cure for 24 hours prior to conditioning, after which the hydrogel is conditioned by alternating ionic strength conditions every 4 hours for at least 3 cycles. This allows the hydrogel to expand and contract to remove unreacted monomers from the hydrogel matrix.

Solutions of PBS were prepared by mixing 9.6 g/L of Dulbecco's phosphate buffered saline in deionized water to make a solution of 165 M PBS. The solution was diluted by mixing 33 mL of PBS with 67 mL deionized water to make 55 mM PBS solution.

Figure 2.2. Glove box used for microsensor hydrogel polymerization. Image A shows the glove box. Image B shows the 365 nm UV light.

2.5 Testing Procedures

Hydrogel samples in this project were tested to determine the pH response time and magnitude. The testing procedures for each experiment will be described in each individual chapter.

2.5.1 Analyte Preparation

The hydrogel samples were tested to determine the average response time and magnitude of the response by alternating pH conditions. PBS solution was prepared as explained previously. Then 0.1 M HCl was added to 100 mL of PBS until a pH level of

7.2 and 7.4 was obtained. The two pH solutions were used to test the pH response of the hydrogel.

2.6 Signal Transduction

2.6.1 Weighing Tests

Hydrogel monoliths were synthesized at 1000 μm in thickness and 1 cm in diameter. The hydrogel samples were each placed into a solution of varying pH. Excess water was removed from each sample, and then each sample was weighed after complete saturation. The hydrogels were then dried in a drying oven at 60 ^{o}C for 12 hours and weighed again. These steps were repeated for 5 cycles to determine both the reversibility of the swelling action and the weight of the solution that was absorbed by the hydrogel sample.

2.6.2 Pressure Sensor Tests

The swelling pressure of the hydrogel samples was measured using two different macro chemomechanical sensors. The first macro pressure sensor is the "M-Biotech" design; the second is the Endevco sensor (Endevco 8510B-2, San Juan Capistrano, CA, USA). Both sensors consisted of a piezoresistive sensor (EPX Series, Measurement Specialties, Hampton, Virginia, USA) and a cap containing a porous mesh membrane (see Figure 2.3). This device encloses the piezoresistive sensor and the hydrogel, while allowing fluid exchange between the exposed surface of the hydrogel and the surrounding environment. The cylindrical sample of hydrogel was placed in the chemomechanical sensor and held in place using the screw-on cap. The porous membrane was a stainless

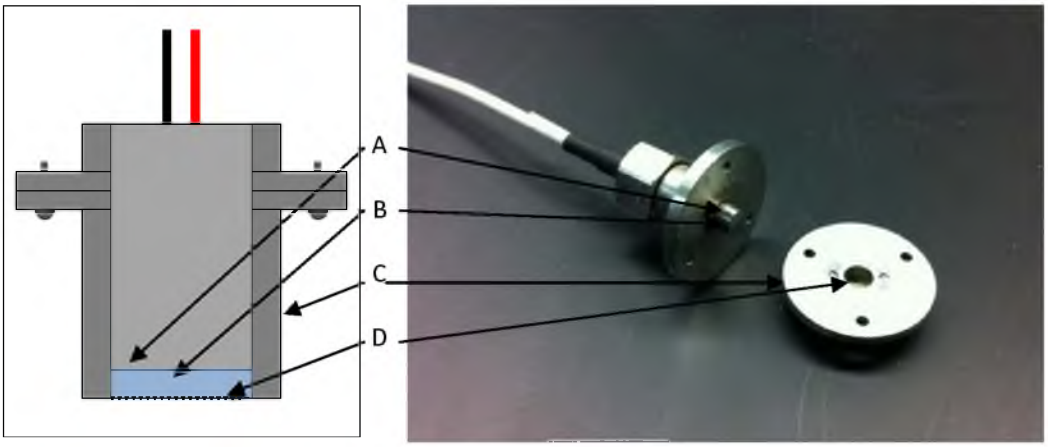

Figure 2.3. The setup for the macro chemomechanical sensor includes a piezoresistive surface pressure sensing surface (A) (the M-Biotech sensor is shown here), a hydrogel sample of 400 um in thickness and 3.5 mm in diameter placed on the sensor (B), a stainless steel sensor cap (C), and a mesh membrane to allow fluid exchange (D).

steel wire cloth mesh (120) from Small Parts, Inc., Logansport, Indiana, USA. The sensor was placed in a stirred temperature controlled bath, and the signal was transmitted to a PC using an Agilent 34970A Data Acquisition Device (Santa Clara, California, USA)

The pressure sensor transduced the change in swelling pressure into a voltage. The voltage measurement was used to determine both the response time and magnitude. The response time was calculated as the time from the initial change in environmental pH to the time at which the hydrogel swelling pressure response reached a stable value (+/- 0.1 mV).

2.7 Continuous Flow Test Platform

A continuous flow system was used in some of the experiments to reduce user error. The continuous flow system utilized LabVIEW software (National Instruments Corporation, Austin, Texas, USA), and is illustrated with a schematic diagram in Figure

2.4. Photographs of the actual system are shown in Figure 2.5, and the descriptions are listed in Table 2.2. The continuous flow system reduced user error by providing a method for fluid exchange without introducing mechanical force to the system. In many of the experiments presented in this project, the media conditions were altered between beakers of solution. This introduced a spike in the sensor output, often accompanied by a drift in the baseline voltage. The continuous flow system mitigated the spike seen and maintained a fixed baseline. In addition to the reduction of user error, the continuous flow system allowed for continuous cycle testing of samples, therefore making it possible to test through over 100 cycles in a 24-hour period.

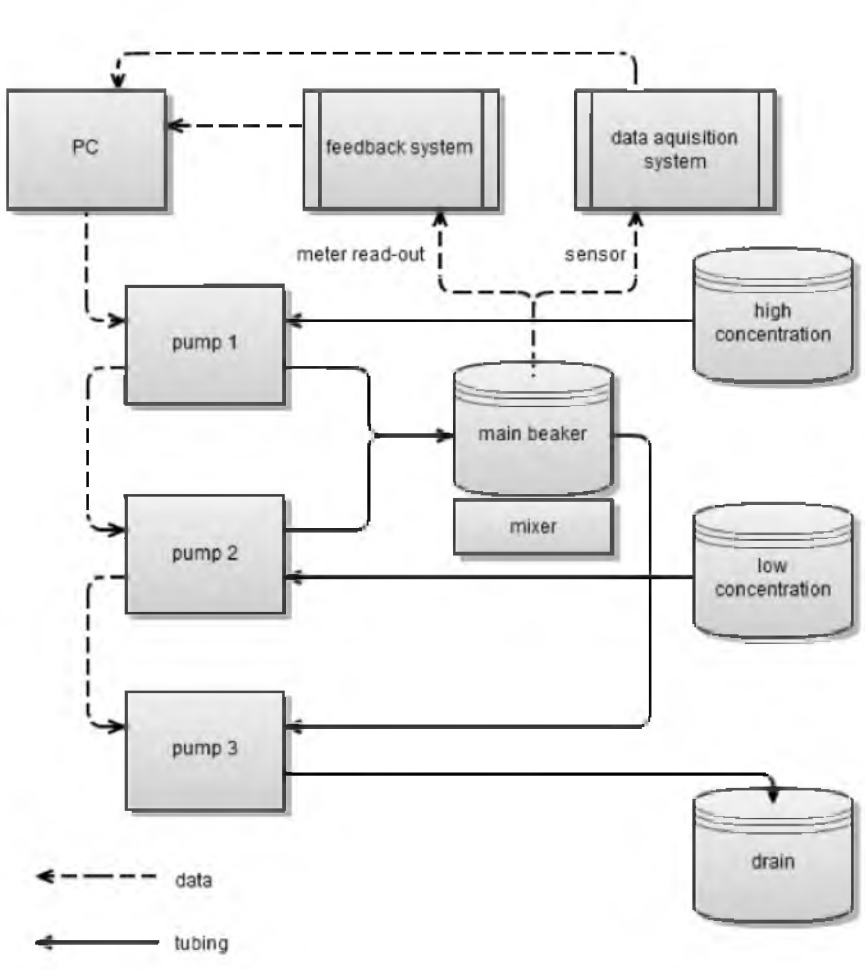

Figure 2.4. Composition and operating flow of the continuous flow test platform

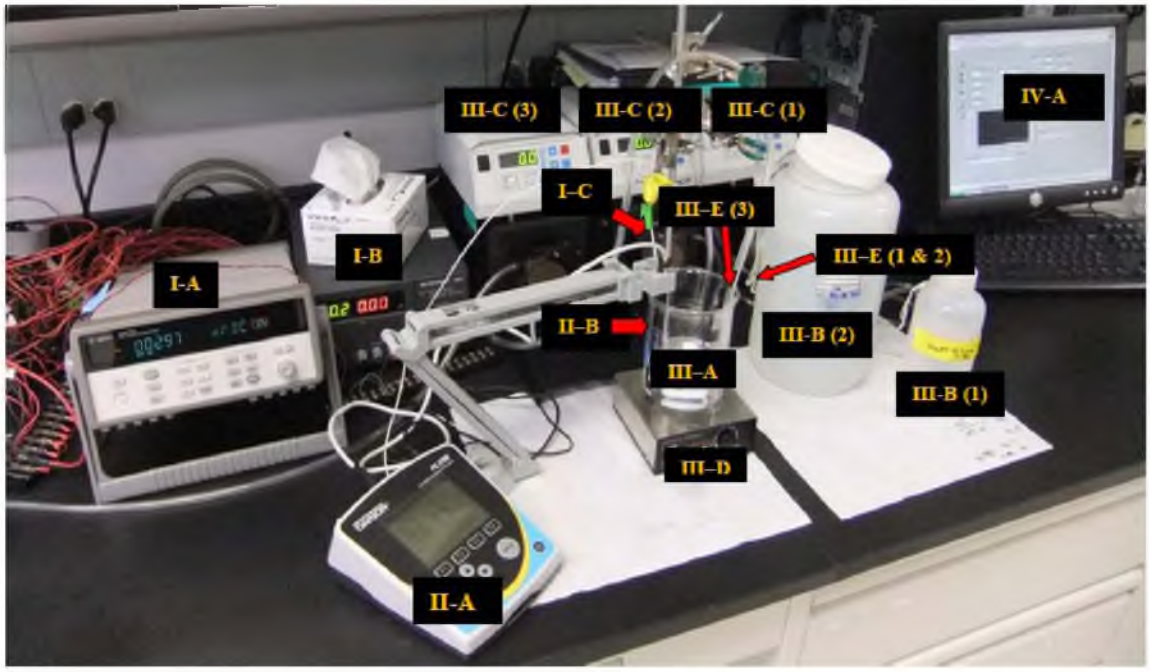

Figure 2.5. The continuous flow platform: I is the piezoresistive pressure sensor system, II is the conductivity measurement system, III is the media solution control system, and IV is the operating system. Each component is described in Table 2.2.

2.8 Data Analysis

2.8.1 Converting the Signal

The signal output was collected in units of mV. The sensitivity of the pressure sensor was characterized with a water column, and the signal was converted to units of Pascals with equation 2.1.

$$P = SV + X \tag{2.1}$$

In this equation, P is the pressure calculated, S is the sensitivity of the sensor used, V is the voltage obtained from the pressure sensor, and X is a scaling parameter based on the baseline data of the sensor obtained prior to sample testing.

Table 2.2. Description of the components labeled in Figure 2.5

	Name	Description
I-A	Data acquisition system	Displays voltage value for the pressure sensor
I-B	Power supply	Power supply for the pressure sensor
I-C	Pressure sensor	Used to measure hydrogel swelling
II-A	Meter display	Displays conductivity, temperature and pH of media solution
II-B	Electrode	Measures the conductivity of the solution
III-A	Main beaker	Contains analyte solution being measured
III-B (1)	Condensed stock solution	Connects to inlet tube 1
III-B (2)	Dilution solution	Connects to inlet tube 2
III-C (1)	Pump 1	Controls condensed stock solution
III-C (2)	Pump 2	Controls dilution solution
III-C (3)	Pump 3	Controls drain
III-D	Magnetic stirring system	Used to mix solutions
III-E (1)	Inlet tube 1	Tube for condensed stock solution
III-E (2)	Inlet tube 2	Tube for dilution solution
III-E (3)	Drain tube	Tube for the main beaker
IV-A	System operating computer	Contains LabVIEW software and system controls

Pressure data from the piezoresistive pressure sensor was analyzed with equation 2.2:

$$R = \ln (P_{eq} - P) \tag{2.2}$$

In equation 2.2, R is the response time, P_{eq} is the equilibrium pressure, and P is the pressure measured at each time interval.

A plot of time vs. R was generated for each experiment. The response time was calculated by equation 2.3:

$$response\ time = \frac{1}{m} \tag{2.3}$$

In equation 3, m is the y-intercept of the plot. The magnitude is calculated by subtracting the smallest measurement from the largest. The magnitude of the response will help determine the sensitivity of the hydrogel to changes in conditions.

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CHAPTER 3

CHARACTERIZATION OF pH-RESPONSIVE 2-HYDROXYETHYL METHACRYLATE HYDROGELS

3.1 Introduction

Hydrogels have proven their ability to respond to changes in the local environment [1-6]. While the results obtained by many researchers highlight the promising nature of hydrogels in biomedical sensors, work has yet to be done to demonstrate the ability of hydrogels to respond to small changes in pH and other analyte concentrations in solution. Some researchers have proposed utilizing hydrogel-based sensors in implantable devices [7-14]. There are others who have focused on hydrogel-based sensors utilizing 2-hydroxyethyl methacrylate (HEMA) hydrogels for use in measuring changes in systemic pH changes in the human body [9-11]. HEMA hydrogels have demonstrated their ability to respond to changes in pH and have a simple swelling mechanism in response to changes in the media solution.

A HEMA hydrogel of the same composition was tested under pH and ionic strength conditions to determine the response time and magnitude to changes in the analyte concentration. The gel was tested to determine the sensitivity to small changes in

pH as well as the cross-sensitivity response to other analytes that may be present under systemic physiological conditions.

3.2 Experimental Methods

3.2.1 Materials

The following monomers were used as received from Sigma Aldrich: 2-hydroxyethyl methacrylate (HEMA), dimethylaminoethyl methacrylate (DMA), tetraethylene glycol dimethacrylate, 2,2-dimethoxy-2-phenylacetophenone (DMPAP), ethylene glycol (EG), and Dulbecco's phosphate buffered saline (PBS).

3.2.2 Hydrogel Synthesis

Hydrogel monoliths were synthesized in this study in a mole ratio of 91.2 DMA, 1.1 HEMA, 0.2 TEGDMA, and 7.5 EG. The pregel solution was injected into a synthesis module and exposed to ultraviolet light at 365 nm for 90 seconds. After polymerization, the hydrogel monolith was washed with deionized water and conditioned with PBS in preparation for experimental testing.

2.2.3 Testing Procedures

The swelling pressure of the hydrogel samples was measured using a pressure sensor [12-14]. The sensor consisted of a piezoresistive sensor and a cap containing a porous mesh membrane. The sample of hydrogel was loaded into the pressure sensor and placed into the testing conditions stated below for experiments with the pressure transducer. The conditions were changed after the pressure reached equilibrium.

3.2.4 Testing Conditions

3.2.4.1 pH-Sensitive Hydrogel Volume Measurement Test

Samples were taken from the hydrogel monolith, measuring 3.5 mm in diameter and 400 μm in thickness. The samples were placed into solutions of varying solutions: bicarbonate buffer, PBS, and sodium/potassium phosphate buffer. The pH was altered between 7.2 and 7.4, and samples were saturated for 24 hours to determine the volumetric swelling change of the gel from the solution. The bicarbonate solution was created by mixing 8.4 g/L to make 0.1 M solution at pH 9.2. Using a pH electrode, 0.1 M HCl was added to the solution to decrease the pH to 7.2 and 7.4. PBS was mixed with 9.6 g/L. At room temperature, the PBS solution had a pH of 7.58. Using a pH electrode, 0.1 M HCl was added to the solution to decrease the pH to 7.2 and 7.4. Sodium/potassium phosphate buffer was mixed according to Table 3.1. After saturation, excess moisture was removed prior to measuring the samples. Each sample was measured with a microcaliper to determine the change in both the diameter and thickness of the hydrogel sample. These tests were repeated three times in each solution. Furthermore, samples were not used for more than one solution.

3.2.4.2 pH-Sensitive Hydrogel Weighing Test

Hydrogel monoliths were synthesized at 1000 μm in thickness and 1 cm in diameter. The gel samples were each placed into a solution of varying pH, from pH 6.0 to 8.0 (see solution compositions in Table 3.2). Excess water was removed from each sample, and then each sample was weighed after complete saturation. The gels were then dried in a drying oven at 60 ^{o}C for 12 hours and weighed again. These steps were

Table 3.1. Phosphate buffer composition for specific pH levels at room temperature from pH 7.0 to 8.0.

Potassium Phosphate Monobasic Anhydrous g/L	Sodium Phosphate Dibasic Heptahydrate g/L	25 °C pH
9.36	32.73	7.0
1.27	50.81	8.0

repeated for 5 cycles to determine both the reversibility of the swelling action and the weight of the solution that was absorbed by the hydrogel sample.

3.2.4.3 pH-Responsive Test in Phosphate Buffer

with a Pressure Transducer

The hydrogel samples were tested to determine the pH response to a buffer using potassium phosphate monobasic and sodium dibasic. The salts were mixed in the concentrations to a 0.2 M solution as described in Table 3.1. This test was performed to determine the response time and pressure response of the gel sample to changes in pH.

The response time was calculated in these tests with the following equation:

$$P=S\ [Pa/mV] * (V+X)\ [V] \quad (3.1)$$

In this equation, P is the pressure calculated, S is the sensitivity of the sensor used, V is the voltage obtained from the pressure sensor, and X is a scaling parameter based on the baseline data of the sensor obtained prior to sample testing. The response magnitude is calculated by subtracting the highest value from the lowest value in each swelling and deswelling cycle. Data presented represent the average values of the response times and magnitudes across the cycles of each test.

3.2.4.4 pH-Sensitivity Step Test from pH 6.0 to 8.0

with a Pressure Transducer

Additional mixtures of the potassium phosphate monobasic/sodium dibasic buffer were mixed to make solutions with the following pH levels in a 0.2 M solution (see Table 3.2). The hydrogel samples were tested to determine the magnitude of the response to small changes in pH and the range at which the response would no longer be separated from the noise of the pressure sensor.

3.2.4.5 Ionic Strength Cross-Sensitivity Test

The hydrogel was tested to determine the response to changes in ionic strength. Hydrogel samples were tested in PBS from 55 mM to 165 mM. The testing solution was

Table 3.2. Phosphate buffer composition for specific pH levels at room temperature from pH 6.0 to 8.0.

Potassium Phosphate Monobasic Anhydrous g/L	Sodium Phosphate Dibasic Heptahydrate g/L	23 °C pH
21.05	6.60	6.0
19.56	9.93	6.2
17.64	14.22	6.4
15.00	20.12	6.6
12.24	26.29	6.8
9.36	32.73	7.0
6.72	38.63	7.2
4.56	43.46	7.4
3.12	46.68	7.6
2.04	49.09	7.8
1.27	50.81	8.0

made with 9.6 g/L of Dulbecco's Phosphate Buffered Saline powder in deionized water. The initial concentration is 165 mM. The solution was diluted with deionized water with 33 mL of 165 mM PBS and 67 mL deionized water to make 1/3X PBS solution. The hydrogel sample was tested in an automated system. The same piezoresistive sensor was used. A conductivity meter was used to monitor the conductivity of the testing solutions, and Lab View software was used to alternate the ionic strength concentrations for the experiment. The test was repeated for 5 cycles.

3.2.4.6 pH Test Performed with Fixed Ionic Strength in Phosphate Buffered Saline

The hydrogel sample was tested in a PBS buffer to determine the response of the gel with fixed ionic strength and varied pH to determine the strength of the isolated pH response. The PBS solution was mixed with 9.6 g/L as in previous experiments, and the pH was altered using a pH electrode and by adding 0.1 M HCl to decrease the pH from 7.58 to 7.4 and 7.2.

3.2.4.7 Glucose Cross-Sensitivity Test

The hydrogel was tested to determine the cross-sensitivity to changes in glucose concentrations at physiological conditions. The hydrogel was tested in the same automated system described above and within a range of 5 mM and 10 mM glucose concentration in PBS.

3.2.4.8 Chloride Ion Sensitivity Test

The hydrogel was tested to determine the response to small changes in isolated ions. A solution was prepared with 0.001 M HCl and 0.0001 M HCl. The hydrogel was loaded into the pressure sensor and tested manually.

3.3 Results

3.3.1 pH-Sensitive Hydrogel Volume Measurement Test

Samples were removed from PBS solution under normal conditions (23 ^{o}C and 9.6 g/L). Each sample was placed in either bicarbonate solution, PBS solution, or phosphate buffer described in Figure 2.4. The samples were altered between pH 7.2 and pH 7.4. Both the diameter and height of the cylindrical samples were measured after complete saturation in each solution, and the volume of the cylinder was calculated using the following formula:

$$V=\pi r^2 h \tag{3.2}$$

In the equation, V represents the volume, r is the radius of the sample, and h is the height/thickness of the hydrogel sample. The initial volume of each sample was measured: d=3.5 mm, h=0.31 mm, v=2.98 mm^3. The results of the change in volume of each solution are given in Table 3.3.

Table 3.3. The volume change from pH 7.2 to 7.4 in each solution.

Solution	ΔVolume	Percent ΔV
Bicarbonate Buffer	1.09 mm^3	54%
Phosphate Buffered Saline	3.95 mm^3	124%
Phosphate Buffer	2.64 mm^3	34%

The hydrogel monoliths were synthesized with a thickness of 400 μm; however, after the initial saturation of the hydrogel, the thickness of the monolith decreased. As the hydrogel samples were tested in each of the conditions for this experiment, the samples further deswelled in the pH 7.4 solutions. The data gathered in this experiment demonstrated that the hydrogel samples do not swell at the same percent in solutions of fixed pH. The results also demonstrated that the hydrogels used in this experiment respond to additional environmental stimuli.

3.3.2 pH-Sensitive Hydrogel Weighing Test

Hydrogel samples were weighed in both the saturated and dried states to determine the weight of the water that was absorbed into each hydrogel sample after saturation. During the test, it was observed that the pH level of the solution had an effect on the physical properties of the hydrogel samples. In this case, the physical change occurred in curling or folding of the sample. As the pH decreased, the degree of folding decreased (see Figure 3.1).

Figure 3.1. Photograph taken of the hydrogel samples after they have been saturated in solutions of varying pH.

The data illustrated in Figure 3.2 demonstrate that the degree of swelling is directly related to the pH of the buffered solution.

3.3.3 pH-Responsive Test in Phosphate Buffer

To determine the pressure change due to the change in pH, hydrogel samples were tested with the piezoresistive pressure (see Figure 3.3). The average swelling pressure exerted on the sensor due to swelling was 32.5 KPa, with a response time of 41 minutes. The average pressure from deswelling was 32.2 KPa, with a response time of 61 minutes. This experiment demonstrated that the degrees of swelling and deswelling pressures are similar; however, the swelling response time was 20 minutes faster than the deswelling time. This experiment also illustrated that the swelling and deswelling of the hydrogel was reversible, and could be used for multiple cycles.

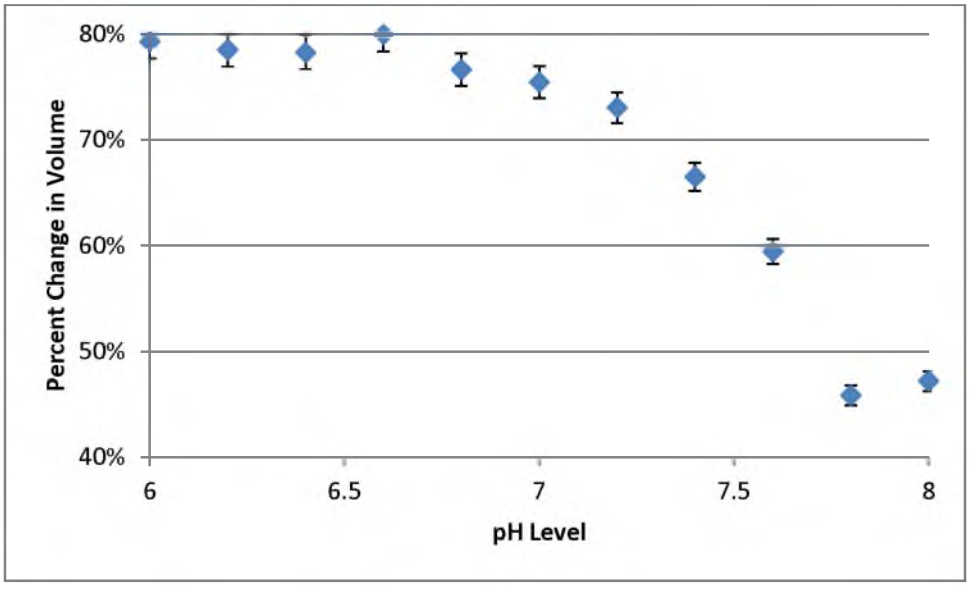

Figure 3.2. Weight change due to the change in pH: as the pH decreases, the degree of swelling increases.

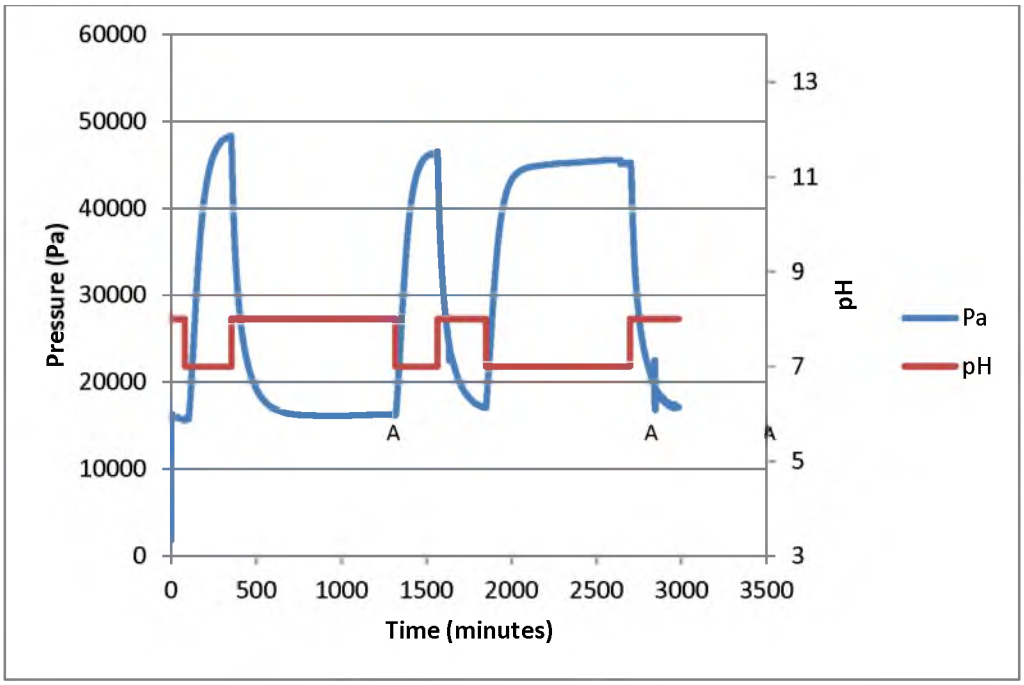

Figure 3.3. The change in the pressure as a result of phosphate buffer from pH 8.0 to 7.0 was tested with a piezoresistive sensor. At points A, the gel was placed in pH 8.0. After the peaks level off, the solution was changed to 7.0.

3.3.4 pH-Sensitivity Step Test from pH 6.0 to 8.0

After determining that the pressure response was measurable with the piezoresistive pressure sensor, another hydrogel sample was tested in increments to determine the response to small changes in pH. This test was also used to determine the pH sensitivity of the hydrogel (see Figure 3.4).

The magnitude of the response at each pH interval and the first order response rate are given in Table 3.4. The data in Table 3.4 show that the maximum sensitivity based on the magnitude of the pressure change is greatest between pH 7.0 and 7.4; however, the response to small fluctuations in pH is detectable in all of the pH ranges tested in this experiment. Furthermore, as the pH increases, the response magnitude

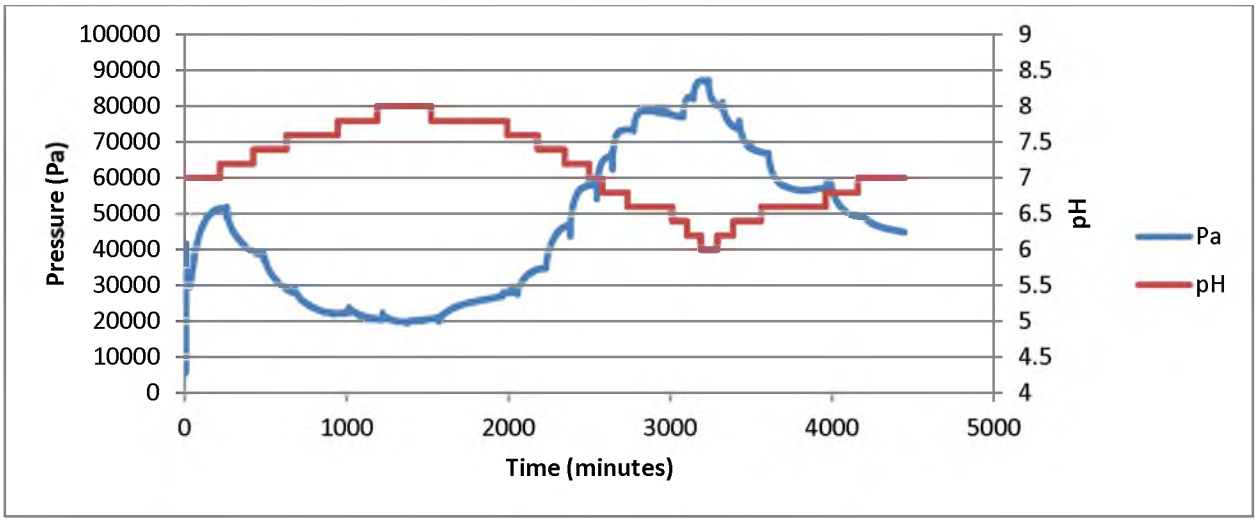

Figure 3.4. The pH response in phosphate buffer from pH 8.0 to 6.0.

Table 3.4. The magnitude of the change in pressure between each pH interval and the response time.

	Swelling		Deswelling	
pH Change	Rate (minutes)	Magnitude (Pa)	Rate (minutes)	Magnitude (Pa)
6.0 - 6.2	7.84	4156.65	16.47	6833.96
6.2 - 6.4	9.67	5528.5	20.2	6684.38
6.4 - 6.6	12.42	7239.49	37.31	8308.54
6.6 - 6.8	6.72	6514.96	40.65	9583.94
6.8 - 7.0	12.3	7320.89	39.37	9651.23
7.0 - 7.2	20.83	10357.7	43.86	12459.19
7.2 - 7.4	28.33	11208.1	43.46	10573.7
7.4 - 7.6	34.84	5876.94	51.81	6365.07
7.6 - 7.8	75.19	4798.87	49.5	3248.62
7.8 - 8.0	58.82	912.87	56.49	2792.04
Averages	26.696	6391.497	39.912	7650.067

decreases from 10 KPa between 7.2 and 7.4 to 2.7 KPa between 7.8 and 8.0. This demonstrates that the sensitivity of the gel begins to decrease as the pH increases.

The pressure and pH response were also plotted on the same graph, which reveals a slight hysteresis (see Figure 3.5). While there is a slight hysteresis to the plot shown in Figure 3.5, the hydrogel still demonstrates a strong reversible response.

3.3.5 Ionic Strength Cross-Sensitivity Test

Since the hydrogel demonstrated a selectivity to different analyte solutions, the hydrogel sample was tested to determine the response sensitivity to changes in ionic strength (see Figure 3.6). The average response time for swelling in this experiment is 19 minutes with a response magnitude of 1.6 KPa. The average time for deswelling is 17 minutes with a response magnitude of 2.8 KPa. This test reveals that the hydrogel composition used in these experiments is not only sensitive to small changes in pH, but also to changes in ionic strength.

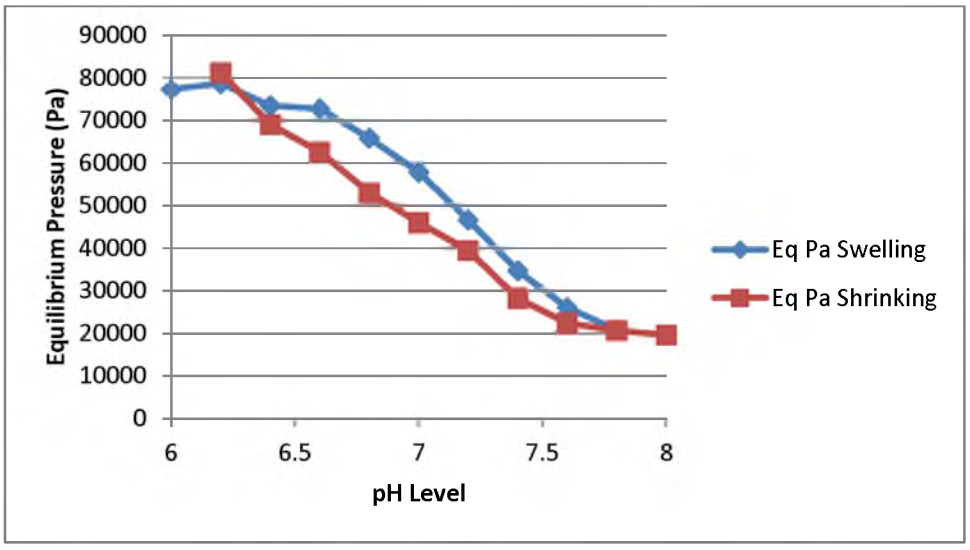

Figure 3.5. Pressure vs. pH reveals a slight hysteresis.

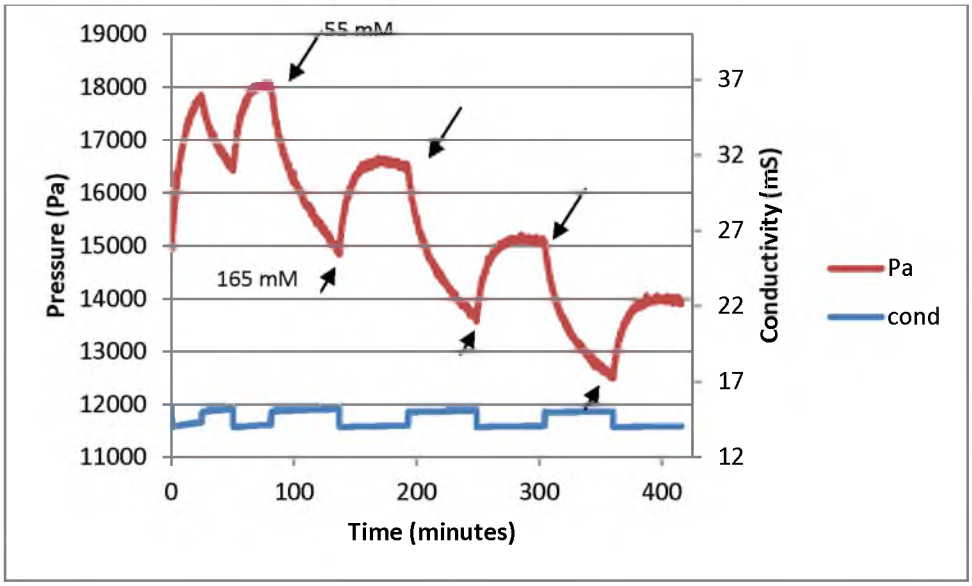

Figure 3.6. An ionic strength test where the conditions were altered between 55 mM and 165 mM PBS

3.3.6 pH Test Performed with Fixed Ionic Strength in Phosphate Buffered Saline

After determining that the hydrogel sample was responsive to changes in ionic strength concentration, an experiment was designed to fix the ionic strength and alter the pH. The hydrogel sample was tested in PBS with fixed ionic strength (165 mM) between pH levels of 7.2 and 7.4 (see Figure 3.7).

The data gathered demonstrate a response to small changes in pH at fixed ionic strength. The response time for swelling is 116 minutes with a magnitude of 2.8 KPa and a response time for deswelling of 83 minutes with a magnitude of 3.2 KPa.

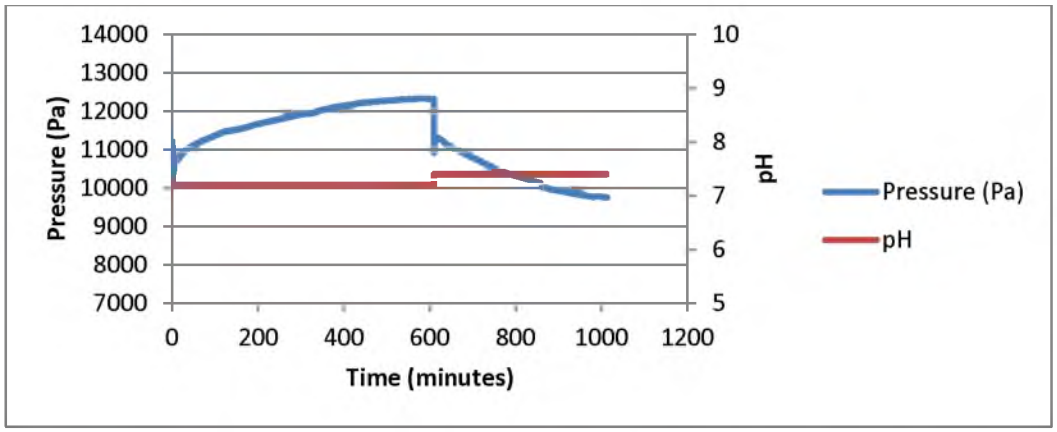

Figure 3.7. The hydrogel response to a pH of 7.2 to 7.4 with fixed ionic strength

3.3.7 Glucose Cross-Sensitivity Test

The hydrogel sample was tested for 5 repeated cycles on the automated system to determine the response to large changes in glucose concentration (see Figure 3.8). There is a small amount of noise in Figure 3.8. Previous studies with the same hydrogel have also shown a large amount of noise due to both the magnetic stirring bar and the movement associated with changing the testing conditions. This test occurred in the automated system, and the noise is likely a result of the fluid exchange both in and out of the system.

3.3.8 Chloride Ion Sensitivity Test

A hydrogel sample was loaded into the piezoresistive sensor and tested under 0.001 M HCl and 0.0001 M HCl conditions. The response is given in Figure 3.9. The response time for swelling is 1.13 minutes with a magnitude of 0.8 KPa and a response time for deswelling of 1.69 minutes with a magnitude of 0.57 KPa, therefore the data demonstrate that hydrogels are responsive to small changes in ion concentration.

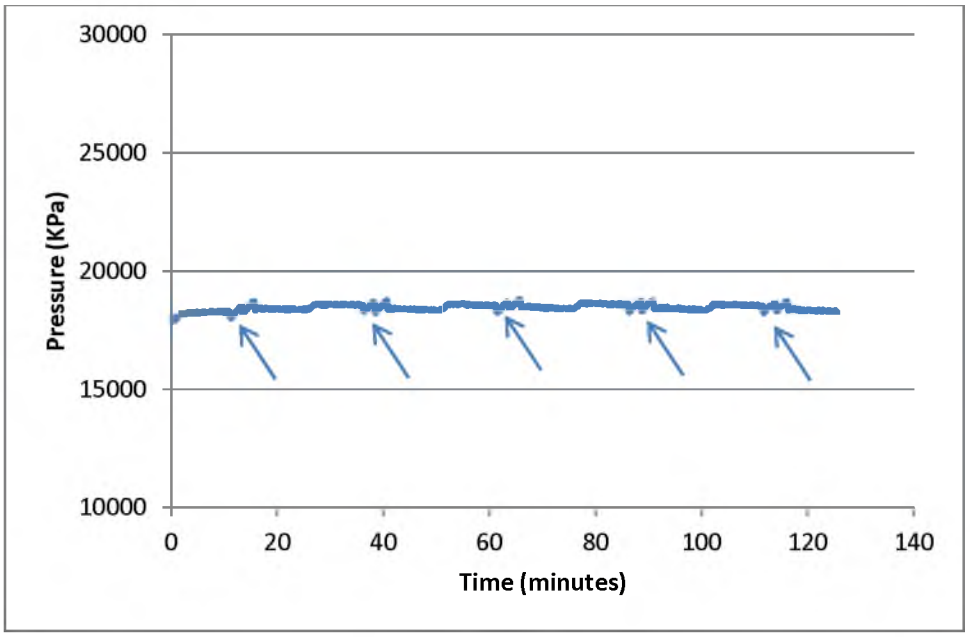

Figure 3.8. The gel sample was tested from 5 mM to 15 mM glucose concentration for 5 cycles. The arrows indicate the beginning of each cycle.

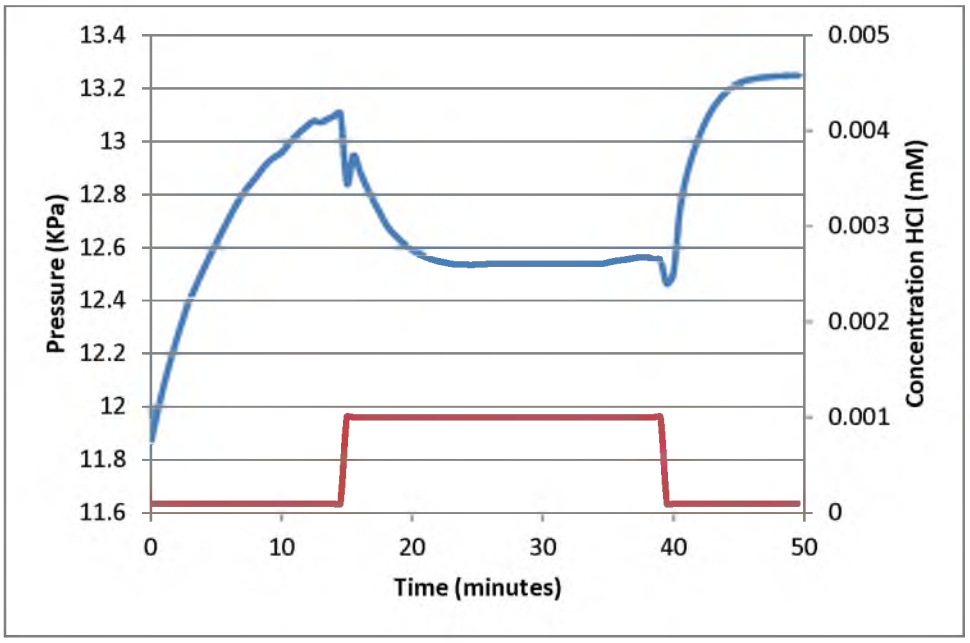

Figure 3.9. Chloride ion sensitivity test from 0.001 M HCl to 0.0001 M HCl

3.4 Discussion

The data gathered in the pH sensitive hydrogel volume measurement test showed that the mechanism for unconfined hydrogel swelling occurs in all directions. This was demonstrated in both the change in diameter and the change in height of the samples. These data help drive the design of devices that are used to measure the change in pressure of the hydrogel samples. If a sensor and cap design includes free space, the hydrogel will swell to fill that area [12]. Therefore, it is important to ensure that the hydrogel is confined to prevent lateral swelling of the hydrogel. By confining the hydrogel within the sensor cap, the hydrogel is able to deflect the piezoresistive diaphragm with uniformity, which enables a more complete signal transduction. Furthermore, the size of the mesh pores is important. If the mesh pores are too large, the hydrogel sample will bulge through the pores, and therefore apply less pressure to the sensing diaphragm [12].

The pH-sensitivity step test demonstrates that the swelling of the hydrogel is directly proportional to the pH of the media solution. As the pH increases, the degree of swelling increases. As the pH approaches 6.0, the plot plateaus. In addition, as the pH approaches 8.0, the magnitude of the response decreases to the point that it may not be easily detected from the noise. The pKa value of the monomers used in this hydrogel falls into the range of 7.0 to 7.2 [9]. Therefore, it is likely that the sensitivity of the hydrogel composition used in this experiment falls within the range of 6.0 to 8.0, which is beyond the regular range for physiological conditions (7.2 to 7.5) [15].

The pH-sensitivity step test was used to determine the hydrogel response to small changes in pH. While the step test illustrates that the hydrogel loses sensitivity as the pH

increases, the plot of pressure versus demonstrates that the response time is reversible with only a slight hysteresis.

Hydrogel samples taken from the same monolith were tested to determine the cross-sensitivity to other analytes. The hydrogel sample was highly responsive, on the order of 1.6 to 2.8 KPa, to changes in ionic strength concentration. The hydrogel response magnitude in the pH sensitive hydrogel volume measurement test demonstrates a varying response to changes in pH with different analyte solutions. The data gathered in the ionic strength cross-sensitivity test demonstrate that the hydrogel responds to changes in ionic strength. Upon further analysis of the solutions used in the pH-sensitive hydrogel volume measurement test, there is a difference in the ionic strengths of the solutions used between pH 7.2 to 7.4. The ionic strength of each solution was calculated using the following formula:

$$I=\tfrac{1}{2}\Sigma cz^2 \tag{3.3}$$

In this equation, I is the ionic strength of the solution, c is the molar concentration, and z is the charge number of each ion in the solution. The ionic strength for potassium phosphate monobasic and sodium phosphate dibasic varies at each pH level. At pH 7.0, the calculated ionic strength was 313 mM and at pH 8.0, it was 388 mM. The difference in the degree of swelling is due to the combined effect of the change in pH and the change in ionic strength of the solutions used. This behavior demonstrates that the swelling response occurs as the side chains on the sensing groups become charged, resulting in electrostatic repulsion. Therefore, hydrogel-based sensors used to measure the change in pH under physiological conditions should consider ionic strength, pH, and other charged interactions together [15].

Hydrogel samples taken from the same monolith were tested to determine the cross-sensitivity to other analytes. The samples demonstrated no response to changes in glucose concentration. The noise seen in Figure 3.8 is a result of fluid exchange and movement of the magnetic stirrer. The noise from the fluid exchange highlighted the points at which the concentration of the solution was changed.

The hydrogel sample was later tested at fixed ionic strength (165 mM) in PBS solution. The pH was decreased to 7.2 and 7.4. The hydrogel was responsive, but the response time was slower, 116 minutes vs. 28 minutes due to the fixed ionic strength conditions. Without the change in ionic strength, which drives a faster response time, the hydrogel response is increased.

The chloride ion sensitivity test demonstrates that the hydrogel sample is responsive to small changes in the concentration of negatively charged chloride ions. This experiment verifies the proposed mechanism for hydrogel swelling. The mechanism states that as nitrogen groups within the hydrogel sensing groups (DMA) are placed in a solution of decreased pH, that the nitrogen group will become protonated and therefore positively charged. This change in the charge causes electrostatic repulsion in the hydrogel backbone, which results in hydrogel swelling [16]. The results of the chloride ion sensitivity test demonstrate an interaction between the positively charged nitrogen groups and the negatively charged chloride ions. As molecules and ions of opposing charge interact with each other, the hydrogel deswells because the charge becomes neutralized. As demonstrated by the experimental results, the hydrogel deswelled as the chloride ion concentration increased.

3.5 Conclusion

Hydrogels with a HEMA back bone structure and DMA sensing groups can be used as stimuli responsive materials. The experiments outlined in this chapter demonstrate the response of hydrogel samples to changes in pH, ionic strength, and changes in ion concentration. Due to the data presented here, hydrogels prove that they are multi-analyte-sensitive materials that can be tested within normal ranges of systemic physiological conditions as well as in bioreactor applications. In addition, the response time for hydrogels is not ideal for continuous systemic monitoring; therefore, future research projects will be designed to optimize the response time.

3.6 References

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CHAPTER 4

HYDROGEL RESPONSE OPTIMIZATION: CHEMOMECHANICAL pH SENSOR RESPONSE TIME AND ITS DEPENDENCE ON COMPOSITION AND THICKNESS

4.1 Introduction

Hydrogels have been proposed as useful materials in biological monitoring [1-17]. Chemomechanical sensors that exploit the hydrogel swelling behavior have been investigated by a number of research groups, but all have noted that the response time is not optimal for continuous system monitoring [1-13]. The response time of stimuli responsive hydrogels has been characterized under a variety of conditions [1-14]. While hydrogels are responsive to small changes in analyte conditions, the response time for hydrogels of 400 μm is greater than 30 minutes [2-5]. In order to utilize hydrogels for continuous monitoring applications, the hydrogel response must fall within a smaller time range, 1 – 5 minutes [15]. Some researchers have proposed that hydrogels with decreased thicknesses will have faster response times, and can therefore be used for continuous monitoring [1-4]. In this study, pH-responsive hydrogels were synthesized

with different thicknesses to determine the effect of decreasing the thickness of the hydrogel on the response time.

After an initial characterization of hydrogels with a 2-hydroxyethyl methacrylate (HEMA) backbone structure (Chapter 3), the response time demonstrated that the hydrogel response did not occur within time constraints that would be conducive to continuous monitoring of conditions in either bioreactors or physiological monitoring. In this chapter, each of the materials used for hydrogel synthesis was analyzed to determine its effect on the hydrogel structure and function. Once the structure and function of each material was defined, an optimized hydrogel with a decreased response time was tested in a series of experiments to determine how the response can be further optimized.

4.2 Experimental Methods

Hydrogel monoliths responsive to pH were synthesized by copolymerizing the monomers dimethylaminoethyl methacrylate (DMA), 2-hydroxyethyl methacrylate (HEMA), and tetraethylene glycol dimethacrylate (TEGDMA) in the nominal mole ratio of 86.1:2.1:0.3. This composition was selected after a series of tests were performed in order to obtain high sensitivity and low response time. The monomers were mixed with a photoinitiator (2,2-dimethoxy-2-phenylacetophenone) in the solvent ethylene glycol, purged with argon gas, and then injected between two glass plates separated by a teflon spacer. Free radical cross-linking copolymerization was initiated by UV irradiation for 90 seconds (365 nm). The monoliths were conditioned prior to testing and loaded into a chemomechanical sensor.

The swelling pressure of the hydrogel in the chemomechanical sensor was measured using a piezoresistive pressure sensor with a cap containing a porous mesh membrane [4-5]. This device enclosed the piezoresistive diaphragm and the hydrogel, while allowing the hydrogel to interact with the fluid of the surrounding environment. A cylindrical sample of hydrogel, 3.5 mm in diameter and thickness between 50 – 400 μm, was inserted in the chemomechanical sensor, which was then placed into the stirred testing chamber. Each sensor response test was performed at a fixed ionic strength of 165 mM and at a fixed temperature of 25 ^{o}C, while the pH of the media solution was cycled between 7.2 and 7.4.

The pressure sensor (Endevco 8510B-2, San Juan Capistrano, CA, USA) transduced the change in swelling pressure into a voltage. The voltage measurement was used to determine both the response time and magnitude. The response time was calculated as the time from the initial change in environmental pH to the time at which the hydrogel swelling pressure response reached a stable value (+/- 0.1 mV).

4.2.1 Composition Changes

The composition of the hydrogel was altered based on several parameters to determine the effect of each composition component on the structure of the hydrogel in all cases, and the stimuli response time in other cases as indicated. The objective of making changes to the composition of the hydrogel was not only to discover the role of each component, but also to optimize the response time of the hydrogels for sensor applications and to maintain a distinguishable signal from the noise from fluctuations in

the environment. All hydrogels used in the following experiments were synthesized with a thickness of 400 μm.

4.2.1.1 Changes to the Amount of Cross-linker

Hydrogels were synthesized with varying concentrations of cross-linker to determine the effect of the cross-linking molecules on the structure of the hydrogel monolith. Experiments were performed with the pressure transducer to determine the effect of altering the amount of cross-linking monomers in the pregel solution. The pressure measurements were analyzed to determine the response time and magnitude.

4.2.1.2 Changes to the Amount of Photo Initiator

Hydrogels were synthesized with varying concentrations of photo initiator to determine the effect of the photo initiator on the structure of the hydrogel monolith. Observations are noted regarding the testability of the resulting hydrogels.

4.2.1.3 Changes to the Ratio of Ethylene Glycol
to the Monomer Concentration

Hydrogels were synthesized with varying concentrations of ethylene glycol to the overall molar ratio of monomers to determine the role of ethylene glycol in the resulting monolith structure. Some researchers have claimed that ethylene glycol is a solvent for the pregel solution and increases the ability for the hydrogel to respond in a pH range of 6.5 to 7.5 due to the pK_a of ethylene glycol [9-11]. In this experiment, the amount of ethylene glycol is analyzed in two different compositions of hydrogels that have

demonstrated their response to changes in pH. These compositions are based on hydrogel compositions used in other research projects [9-12]. One project used HPMA as the backbone molecule and the other uses HEMA as the backbone. Both hydrogels were tested with the pressure sensor and results were compared to determine the response time and magnitude.

4.2.1.4 Changes to the UV Exposure Time

Some changes in the degree of polymerization were noted in previous experiments. Due to these observations, a series of hydrogels were synthesized using varying ratios of HPMA and DMA to determine the amount of time needed to create hydrogel monoliths that could be used for testing with the piezoresistive pressure sensor. The composition of these hydrogel samples is given in Table 4.1.

4.2.1.5 Changes to the Backbone-Sensing Group Ratio

After determining the effects of the amount of cross-linker, photo initiator, and solvent on the composition of the hydrogel, a series of hydrogel monoliths were synthesized with varying concentrations of backbone monomers (HEMA) and sensing groups (DMA) to the remaining monomers of the pregel solution. The volume of HEMA and DMA was fixed to 145.8 μL, based on previous experiments. The amount of HEMA and DMA was increased and decreased to determine the ratio that would provide that fastest response time. The hydrogel identification and composition of HEMA gels are given in Table 4.2.

Table 4.1. An identification of different hydrogel compositions with varied ratios of DMA and HPMA.

Gel ID	HPMA (μL)	DMA (μL)	TEGDMA (μL)	Ethylene Glycol (μL)	DMPAP (mg)
HPMA D1	500	258	31.2	200	14
HPMA D2	371	387	31.2	200	14
HPMA D3	242	516	31.2	200	14
HPMA D4	113	646	31.2	200	14
HPMA D5	0	758	31.2	200	14
HPMA D6	629	129	31.2	200	14
HPMA D7	672	86	31.2	200	14
HPMA D8	694	65	31.2	200	14
HPMA D9	707	52	31.2	200	14
HPMA D10	758	0	31.2	200	14

Table 4.2. An identification of different hydrogel compositions with varied ratios of DMA and HEMA.

Gel ID	DMA (μL)	HEMA (μL)	TEGDMA (μL)	Ethylene Glycol (μL)	DMPAP (mg)
HEMA D1	72.9	72.9	34.4	273.9	28.9
HEMA D2	109.4	36.5	34.4	273.9	28.9
HEMA D3	145.8	0	34.4	273.9	28.9
HEMA D4	36.5	109.4	34.4	273.9	28.9
HEMA D5	18.2	127.6	34.4	273.9	28.9

A weighing test was used in this experiment. The hydrogel monoliths were placed in solution of PBS with a pH of 7.2 and 7.4. Each hydrogel was wiped of excess moisture and weighed 24 hours after placement into the solution of pH 7.4 to determine the initial weight. Each hydrogel sample was then placed into a solution of pH 7.2 and weighed after 15-minute time intervals until the hydrogel response had reached equilibrium, which was noted as eight continuous weight measurements with no change.

The final weight was recorded 24 hours after being placed into the pH 7.2 solutions for each hydrogel sample to ensure there was no additional response after the weight measurements had stopped. After the conditions for pH 7.2 were recorded, the hydrogels were placed back into the pH 7.4 solutions and the same procedures were followed. The only exception to the procedures was the observation that some of the hydrogel samples responded faster than anticipated, so the time interval was adjusted to 10 minutes until the hydrogel samples reached equilibrium.

4.2.2 Changes in Hydrogel Porosity

The hydrogel composition HEMA D4 was perforated 50 times with a sharp tip to determine the change in the response time and magnitude. The hydrogel sample was loaded into the pressure sensor and the swelling and deswelling responses were collected. The thickness of the hydrogel sample used in this experiment was 400 μm.

4.2.3 Changes in Hydrogel Thickness

Hydrogels were synthesized with the composition of HEMA D4 and were tested with the pressure sensor to determine the effect of the thickness of the hydrogel sample on the response time. Hydrogel samples were synthesized in the following thicknesses: 400 μm, 200 μm, 100 μm, and 50 μm.

4.3 Results

4.3.1 Composition Changes

4.3.1.1 Changes to the Amount of Cross-linker

Hydrogels synthesized with a higher ratio of TEGDMA were rigid. The swelling response rate for the initial composition was 33 minutes with a response magnitude of 18.5 KPa, while the response rate for the composition with 75% of the original composition of TEGDMA was 39 minutes with a magnitude of 71.1 KPa. The response time was longer for hydrogel samples with a decreased cross-linking of the matrix.

4.3.1.2 Changes to the Amount of Photo Initiator

Hydrogels synthesized with a higher ratio of DMPAP were rigid, but also brittle and had an increased adhesion of the glass plates and were difficult to remove from the synthesis module. The initial weight of DMPAP was decreased by 50%, which resulted in hydrogels that were less brittle than could be tested.

4.3.1.3 Changes to the Ratio of Ethylene Glycol to the Monomer Concentration

A hydrogel sample was prepared without ethylene glycol. The resulting hydrogel was rigid, brittle, and could not be loaded into the pressure sensor. Therefore, ethylene glycol is an important part of the composition because it makes the hydrogel more pliable.

A series of hydrogels with varying monomer compositions was synthesized. Table 4.1 outlines a series of hydrogels based on a HPMA backbone, while Table 4.2

includes HEMA hydrogels. The difference between the molecules is only one carbon atom; therefore, the two hydrogel backbones are similar enough to compare the results. The part of the composition that is most different is the ratio of monomers (HEMA/HPMA and DMA) to ethylene glycol. The data shown in Figure 4.1 demonstrate the long response time and the large magnitude of HPMA hydrogels.

The data shown in Figure 4.2 demonstrate the shorter response time of HEMA hydrogels and the increased magnitude. The data from these two experiments demonstrate that ethylene glycol is an important part of the composition because it makes the gels less brittle and rigid and may also increase the sensitivity of the hydrogels. In

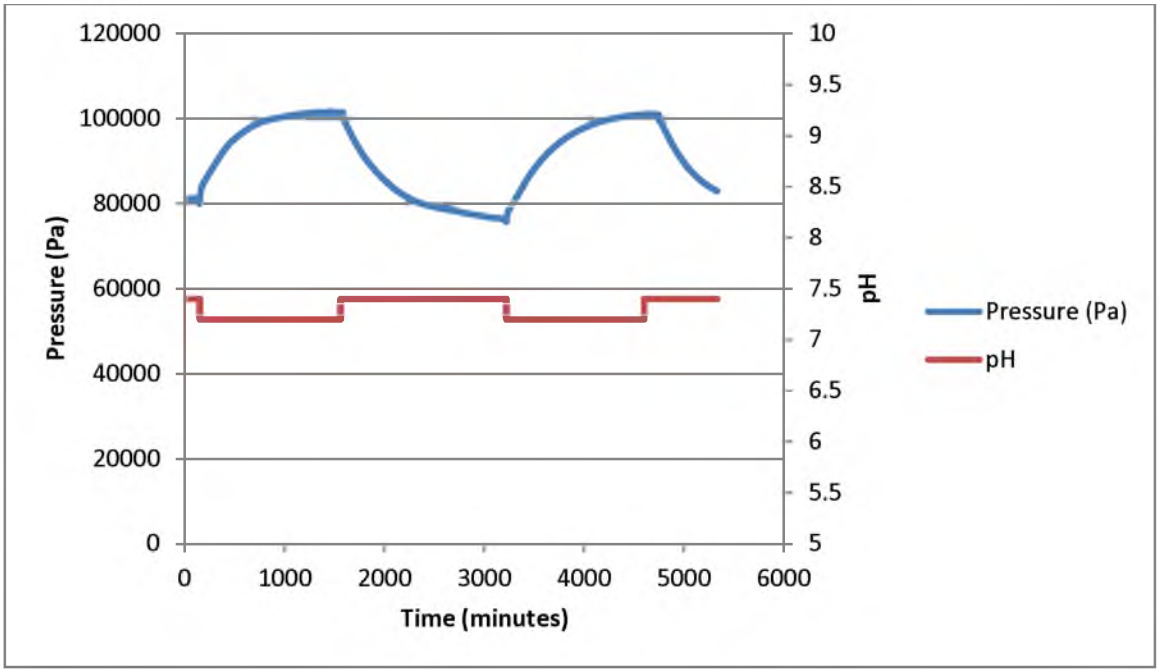

Figure 4.1. The pH response of HPMA hydrogels tested from pH 7.2 to 7.4. The response time for swelling is 238 minutes with a magnitude of 24 KPa and a first order response time for deswelling of 416 minutes with a magnitude of 26 KPa.

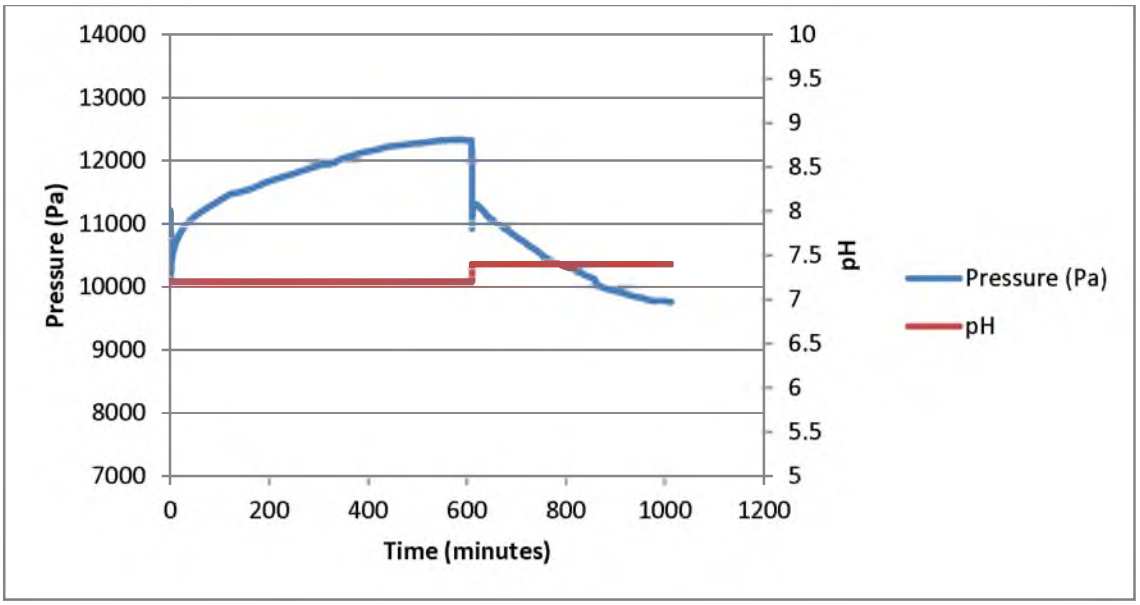

Figure 4.2. The pH response of HEMA hydrogels tested from pH 7.2 to 7.4. The response time for swelling is 116 minutes with a magnitude of 2.8 KPa and a first order response time for deswelling of 83 minutes with a magnitude of 3.2 KPa.

addition, the increased concentration of ethylene glycol has proven across multiple experiments to decrease the response time of the hydrogel samples.

4.3.1.4 Changes to the UV Exposure Time

During the synthesis of the HPMA and HEMA hydrogels, it was observed that the UV exposure time was different for each hydrogel composition, dependent upon the ratio of backbone to DMA. Hydrogels that were exposed to UV for less time were still fluidic, while hydrogels exposed to UV for more time were rigid. Both fluidic and rigid hydrogels were not testable in those states. Due to this observation, pregel solutions were injected into the synthesis module and exposed to UV light at intervals until the needed modulus was obtained. The results of the necessary UV exposure time and the percentage of HPMA to DMA are given in Figure 4.3. Hydrogels with an equal ratio of

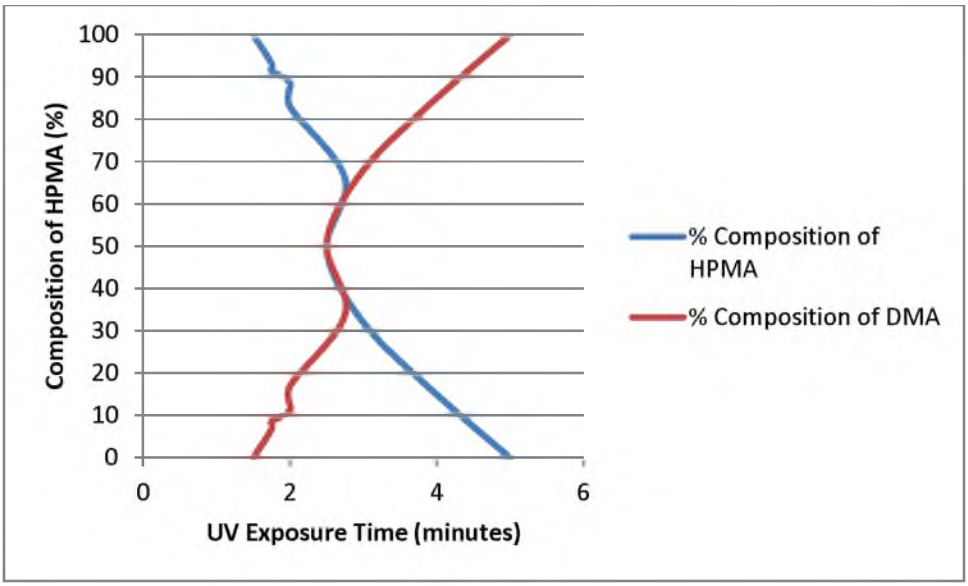

Figure 4.3. The UV exposure time vs. composition % of HPMA to DMA.

backbone to DMA had a shorter UV exposure time than those with higher concentrations of either backbone monomers or DMA. An increased concentration of DMA resulted in an increased UV exposure time, while an increased concentration of backbone monomers resulted in a decreased exposure time.

4.3.1.5 Changes to the Backbone-Sensing Group Ratio

The hydrogel samples prepared for the composition experiments were tested using weight testing methods to determine the response time of the various concentrations. The hydrogel samples varied slightly in some cases and greatly in other with respect to the response time and the degree of swelling. While there were many hydrogel samples tested, the results of five of the hydrogel samples are given in Figure 4.4. The remaining hydrogels were not plotted because their results are not of interest.

Hydrogels with an increased DMA to backbone (whether HEMA of HPMA) show an increased sensitivity and a decreased response time. Therefore, an increase in the amount of sensing group monomers results in a tradeoff where the sensitivity of the hydrogel is sacrificed for the sake of decreasing the response time. Validation tests were performed on HPMA D1 and HEMA D4 with different sensors. The results for these tests are given in Figure 4.4.

The response for HEMA D4 showed the most promise with respect to a decreased response time, and subsequent tests were performed on HEMA D4 to further optimize the response.

4.3.1.6 Changes in Hydrogel Porosity

After obtaining validation data from the pressure sensor for the HEMA D4 response, the hydrogel sample was perforated 50 times to determine whether the time of diffusion could be decreased due to an increased porosity of the hydrogel sample. The results of this experiment are given in Figure 4.5. As it is demonstrated, the response time was altered slightly when compared to the response time and magnitude given in Figure 4.4, where the response time for swelling of the nonperforated hydrogel is 116 minutes with a magnitude of 2.8 KPa and a first order response time for deswelling of 83 minutes with a magnitude of 3.2 KPa. While the response time only increased by a small degree, the magnitude of the response increased by nearly double. The increased porosity of the hydrogel sample increased the response magnitude, and therefore increased the sensitivity, while only slightly increasing the response time.

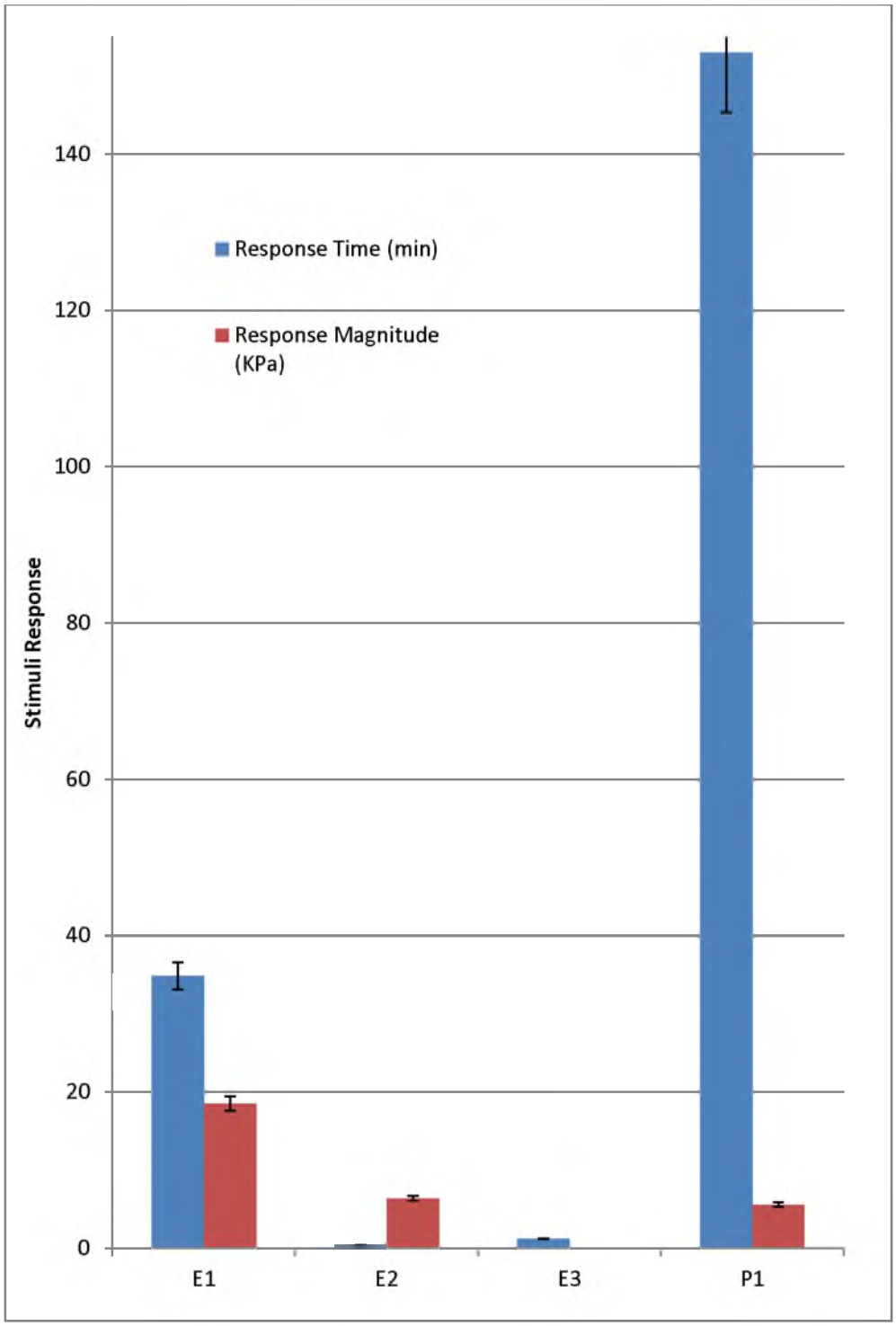

Figure 4.4. Hydrogel composition response time

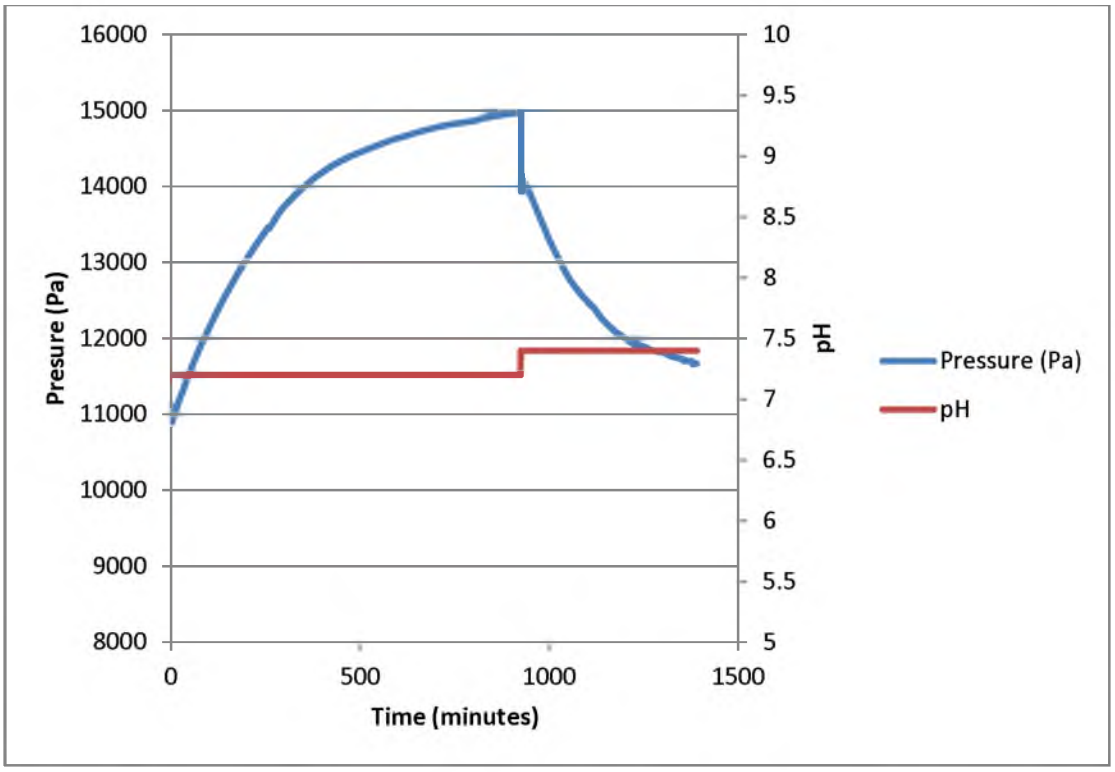

Figure 4.5. The results of a perforated hydrogel test where the first order response time for swelling is 181 minutes with a magnitude of 4.1 KPa and a first order response time for deswelling of 109 minutes with a magnitude of 3.6 KPa.

4.3.1.7 Changes in Hydrogel Thickness

The results comparing the response times for hydrogels of varying thickness are given in Figure 4.6. The response time of the hydrogel is seen to decrease dramatically as the thickness of the hydrogel decreases.

In addition to the decrease in response time, the magnitude of the response also decreases as the hydrogel thickness decreases, though the decrease in magnitude is much more modest. This behavior demonstrates that there is a tradeoff of the sensitivity of the hydrogel as the response time decreases. This was noted by Guenther et al. [18], who explained that as the thickness of hydrogels decreases, the response magnitude decreases. This probably occurs due to loss of signal associated with deflection of the piezoresistive

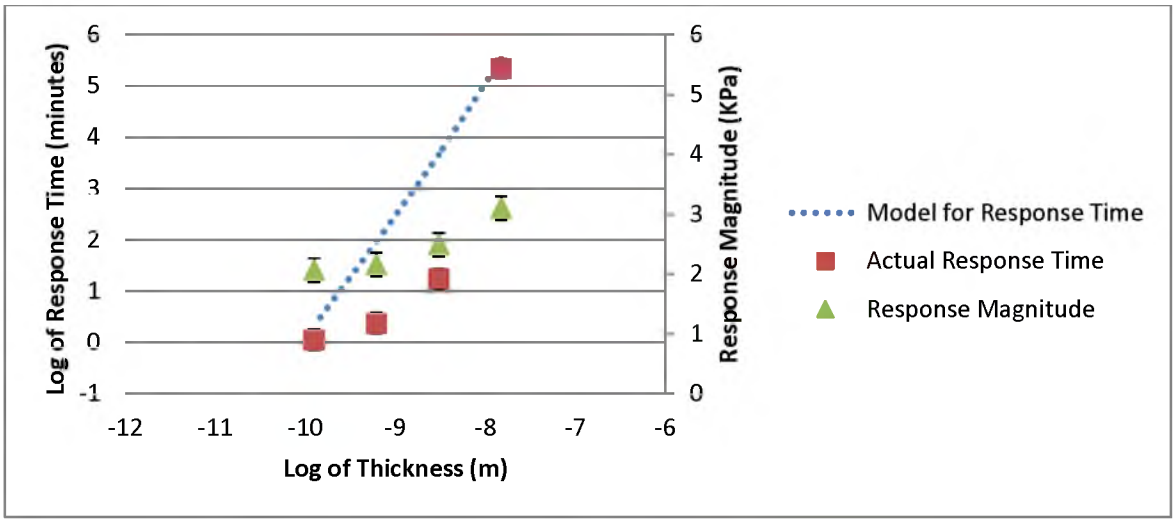

Figure 4.6. Magnitude of the sensor response and natural logarithm of the sensor response time vs. logarithm of the hydrogel thickness for a pH change between 7.2 and 7.4. The dashed line gives the predicted dependence of the response time for mass-transfer control [18].

membrane, which increases in relative importance as the hydrogel gets thinner. While this is the case, experimental results presented here maintain a measurable difference between small changes in pH; hence, the overall sensitivity of the hydrogel has not been compromised, and thin hydrogel samples can be used to measure small changes in environmental conditions.

4.4 Discussion

Changes to the concentrations of cross-linker monomer and photo initiator of the pregel solutions affected the mechanical properties of the resulting hydrogel samples. Samples prepared with a decreased amount of cross-linker or photo initiator resulted in either loose hydrogels or no hydrogel at all. Samples prepared with a high concentration of cross-linker or photo initiator were rigid and brittle, resulting in hydrogels that could not be tested.

Investigations into the effect of photo initiator on the resulting hydrogel properties demonstrated that the higher the photo initiator concentration, the more brittle the hydrogel, regardless of the monomer composition. Consequently, hydrogels prepared with less photo initiator required a longer UV exposure time, and resulted in hydrogels that were pliable and could withstand greater tolerances in handling the samples. Therefore, the amount of photo initiator has an effect on the degree of polymerization of the hydrogel sample. The increased concentration of photo initiator in the pregel solution results in a higher concentration of polymerization initiation sites, shorter polymer chains and increased brittleness. Conversely, hydrogels prepared with a decreased concentration of photo initiator have a fewer initiation sites, take longer to propagate, and result in longer polymer chains and therefore increased pliability [20].

The ratio of ethylene glycol to backbone monomer is an important consideration with the mechanical properties as well. There is a balance between the concentration of ethylene glycol and the backbone monomers, which has been optimized in the composition tests.

In addition to the ratio of ethylene glycol to backbone monomer, there is a tradeoff between the concentrations of sensing monomers to that of the backbone monomer. When the concentration of sensing monomers is increased, the sensitivity increases, but the response time also increases. This is a result of a higher concentration of branches that may become charged during the swelling and deswelling of the hydrogel. In addition, there is an effect on the diffusion of ions through the hydrogel due to the electrostatic interactions of molecules passing through the hydrogel matrix, which decreases the response time. Therefore, it is important to consider the ratio of backbone

monomer to sensing monomer to allow for faster diffusion through the hydrogel matrix [1,18].

The goal of increasing the porosity of the hydrogel sample was to increase the rate of diffusion through the hydrogel matrix. While the response time was not decreased, the sensitivity of the hydrogel was increased by nearly 25%. This further confirms that the diffusion of ions and other charged particles through the hydrogel matrix is inhibited or enhanced due to the electrostatic interactions of charged molecules passing through the charged polymer matrix. When the ability to diffuse through the matrix is enhanced, ions can enter more freely, therefore creating a charge on a higher concentration of side chains in the hydrogel matrix [1,18].

The UV exposure time was analyzed as a function of concentration. This result held constant as the gels of altered thickness were synthesized. As long as the composition remained constant, the UV exposure time did not change.

Results given by Herber et al. show that pH-sensitive hydrogels of 200 μm in thickness reached an equilibrium response in a chemomechanical sensor within 12 minutes [2-3], as compared to 3.47 minutes for the results presented here. However, the experiments in References 2 and 3 were not performed at fixed ionic strength.

The thickness of the hydrogel samples has an effect on the diffusion through the matrix. Assuming that the sensor response is under mass transfer control, the response time should depend on hydrogel thickness as given by reference 19, which states that the hydrogel response is driven by diffusion; therefore, if the diffusion is constant across the hydrogel, then the response time should depend on the second power of the hydrogel thickness. A comparison of the experimental data and the model is given in Figure 4.6.

The observed dependence on thickness is stronger than predicted by the model for the thicker gels. The model presented in reference 19 is a scaling model that only considers diffusion and thicknesses as factors that affect response time. Simulations of pH-responsive hydrogels based on the Nernst-Planck equation have also been performed [1]. These simulations show that the response time increases with gel thickness, and that the dependence is stronger than quadratic for thicknesses between 150 μm and 300 μm, in agreement with results presented here. The explanation proposed in Reference 1 is that transport of ions within a hydrogel matrix is limited to the regions containing fluid, and that the network chains are impenetrable to ions, increasing the path length that an ion must travel. In hydrogels of decreased thickness, there is a decrease in the number of chains that obstruct the diffusion pathway. This group further proposes that, while ion transport through a hydrogel matrix is a diffusion limited process, there are other factors to affect the rate of ion transport, including electrostatic interactions, electro-diffusion, hydraulic permeability, and fluid pressure gradient. Therefore, factors concerning the structure as well as the properties of functionalized groups on the hydrogel play a role in the diffusion through the hydrogel matrix, which will affect both the response time and magnitude.

4.5 Conclusion

The components used in synthesizing hydrogel samples were analyzed in this chapter to determine their effect on the optimization of the hydrogel stimuli response. The degree of cross-linking and polymerization is directly impacted by the concentrations of cross-linking monomers or photo initiator. Ethylene glycol is more than just a solvent

for the monomers used in synthesizing hydrogels; it is also a necessary component that creates ideal mechanical properties for testing hydrogels in the pressure sensor. The ratio of DMA to backbone monomers will directly affect the stimuli response time and sensitivity. The sensitivity of the hydrogel matrix will decrease as the response time decreases. Therefore, optimization of the stimuli response must consider both the composition and thickness of the hydrogel sample. The response time of a pH-responsive hydrogel decreases with decreasing thickness. The dependence on thickness is stronger than quadratic for thick gels, but much weaker than this for gels thinner than 100 μm. The magnitude of the hydrogel response in a chemomechanical sensor also decreases as the thickness decreases, but the dependence is weaker than linear. Therefore, sensor response time can be reduced significantly by using thin hydrogels without compromise of the ability to detect small pH changes.

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CHAPTER 5

STORAGE AND OPERATIONAL STABILITY OF pH-RESPONSIVE HYDROGELS

5.1 Introduction

Hydrogels have proven their ability to respond to changes in the local environment [1-6]. While the results obtained by many researchers highlight the promising nature of hydrogels in biomedical sensors, work has yet to be done to demonstrate the ability of hydrogels to maintain a response after being stored for an extended period of time, and to demonstrate the ability to maintain a strong stimuli response after repeated cycles. Some researchers have proposed utilizing hydrogel-based sensors in implantable devices [7]. If this technology is to work, it is important to understand the duration and stability of the stimuli response. This will determine the life of a hydrogel-based sensor and the time frame in which the device will become ineffective and need to be replaced. Furthermore, devices may not be used as soon as the hydrogel has been synthesized. Therefore, it is also important to understand how long a device may remain in storage before it loses its effectiveness.

In this chapter, a hydrogel with a 2-hydroxyethyl methacrylate (HEMA) backbone was studied to determine the ability of the hydrogel to respond after extended periods of

time in ambient conditions. The time intervals for this study were at 0, 9, and at 18 months. The data gathered in this chapter will be useful in determining storage duration and conditions for maintaining a strong stimuli response of the hydrogel.

This chapter also addresses the operational stability of the hydrogel. This will help researchers determine the length in which a hydrogel-based chemomechanical sensor may be used in medical and other biological applications without losing sensitivity to changes in environmental conditions.

Here data are presented that have been gathered at set time intervals (0 months, 9 months, and 18 months after hydrogel synthesis) and with prolonged testing (up to 300 cycles). The tests performed on the HEMA hydrogel were under ionic strength conditions. HEMA hydrogels are known to respond to multiple analytes, including pH and ionic strength [8-11]. From the data presented in Chapter 3, the ionic strength response is fast (3-5 minutes), and shows a clear stimuli response to small changes in ionic strength concentrations. Furthermore, the ionic strength response is used here for rapid cyclic testing.

5.2 Experimental Methods

5.2.1 Materials

The following monomers were used as received from Sigma Aldrich: 2-hydroxyethyl methacrylate (HEMA), dimethylaminoethyl methacrylate (DMA), and tetraethylene glycol dimethacrylate. In addition, 2,2-dimethoxy-2-phenylacetophenone (DMPAP), a photoinitiator, and ethylene glycol (EG), a solvent for the pregel solution, were also obtained from Sigma Aldrich and used as received. Dulbecco's phosphate

buffered saline was also obtained from Sigma Aldrich and mixed at 9.6 g/L in deionized water.

After preparation, hydrogel samples were tested with a piezoresistive sensor. A conductivity meter was used to measure the conductivity of the testing solution. An automated, continuous flow system comprising of a data acquisition device, pumps, and lab view software was used to change the concentration of the testing solution.

5.2.2 Hydrogel Synthesis

Hydrogel monoliths were synthesized in a mole ratio of 91.2 DMA, 1.1 HEMA, 0.2 TEGDMA, and 7.5 EG and a thickness of 400 μm. The hydrogel was conditioned by alternating concentrations of PBS every 4 hours for 3 cycles. The PBS concentrations were alternated between 55 mM and 165 mM PBS.

5.2.3 Testing Conditions

This hydrogel has been proven to swell in response to changes in ionic strength. The two testing conditions for the ionic strength test were from 155 mM PBS to 165 mM PBS. To obtain these concentrations, 9.6 g of PBS in powder form was added to 1 L deionized water and diluted to 155 mM and 165 mM concentrations.

5.2.4 Testing Procedures

The swelling pressure of the hydrogel samples was measured using a pressure sensor [12-14]. The pressure consisted of a piezoresistive sensor and a cap containing a porous mesh membrane. The cylindrical hydrogel sample (3.5 mm diameter and 400 μm

height) was placed in the pressure sensor and placed into the testing conditions, starting at 155 mM. The continuous flow equipment was programmed to alternate the concentrations of PBS between 155 mM and 165 mM every 15 to 30 minutes.

5.2.5 Storage Stability

A shelf life test was designed to determine how long a hydrogel sensor could sit on a shelf in a clinic before it would no longer work. For this test, a hydrogel monolith was synthesized, and samples were tested at these time intervals: 1 week after synthesis, 9 months, and 18 months. Hydrogel samples tested at each time interval were performed under ionic strength conditions where the ionic strength of the media solution was changed between 155 mM and 165 mM. Experiments were performed for at least 3 cycles and the average values for the response time and magnitude were collected and analyzed.

5.2.6 Transportation and Signal Stability

Simulated transportation tests were performed on the hydrogels to determine the signal stability after transportation. Two hydrogel monoliths were prepared. The first monolith, the control sample, was synthesized and immediately hydrated, washed, and conditioned as specified above. The second hydrogel monolith was synthesized and immediately placed in a 100 mL container. The container with the hydrogel sample was packaged in a padded mailing envelope and placed in a vehicle for 7 days and driven under normal conditions to simulate travel conditions. The hydrogel was then hydrated, washed, and conditioned as described above.

Both hydrogel samples were tested with the same conditions: 25 °C and 155 mM ionic strength. Solutions for this test were prepared by mixing 100 mL PBS solution with 0.1 M HCl to obtain pH levels of 7.2 and 7.4. The solutions were mixed by adding 500 μL of 0.1 M HCl under constant stirring with a calibrated pH electrode until the necessary pH readings were obtained for each solution.

The hydrogel samples from each monolith were tested with the same sensor for three cycles to determine the change in the response after simulated travel conditions.

5.2.7 Operational Stability

The second test was performed to determine if the stimuli response would decrease after multiple cycles of testing. The hydrogel samples were loaded and tested continuously in the pressure sensor with the automated flow system for up to 100 cycles under the same conditions listed above. The hydrogel samples in each experiment were stored in 165 mM PBS solution at room temperature for up to 18 months.

5.3 Results

5.3.1 Storage Stability

After performing the same test on hydrogel samples taken from the same hydrogel monolith, the data were analyzed to determine the first order response time and the magnitude of swelling.

The following graphs illustrate one swelling and one deswelling cycle at each of the specified time intervals. The arrows on the graphs (see Figures 5.1-5.3) represent changes in the ionic strength concentration during the experiments. The hydrogel

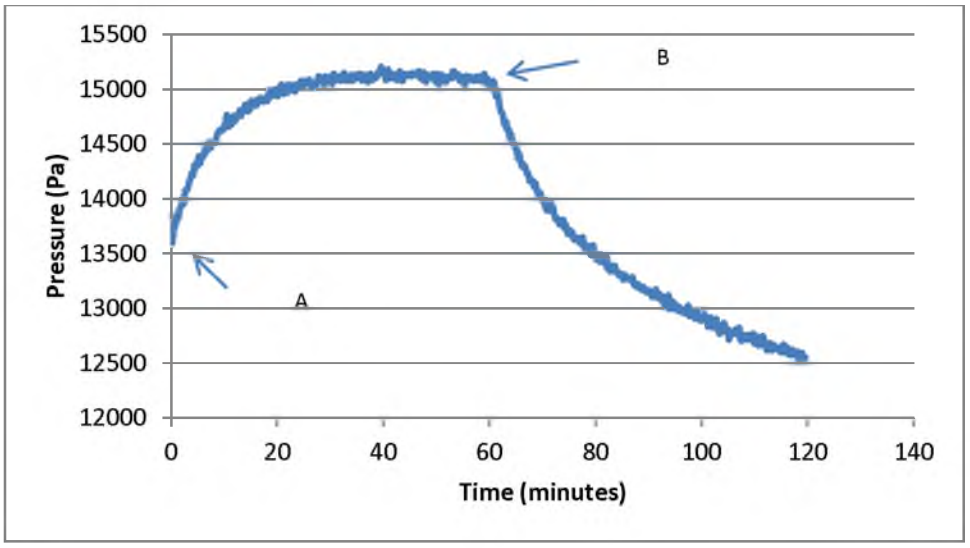

Figure 5.1. An ionic strength test where the ionic strength concentration was decreased (from 165 mM to 155 mM) at point A. When the hydrogel sample came to equilibrium, the concentration was changed to a higher concentration at point B (from 155 mM to 165 mM). The average first order response time was 22 minutes for swelling and 17 minutes for deswelling. The average magnitude response change was 1.6 KPa for swelling and 2.8 for deswelling.

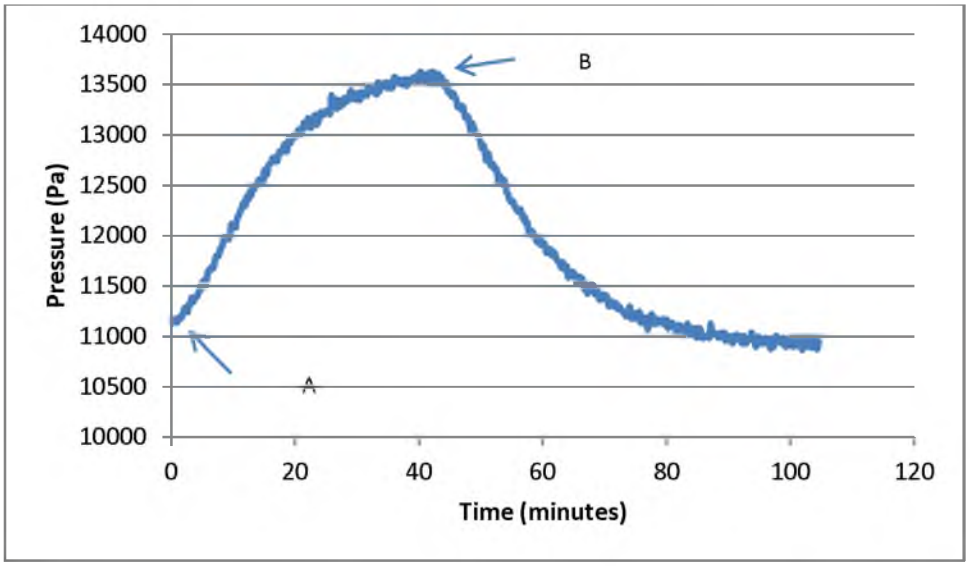

Figure 5.2. An ionic strength test after 9 months where the average first order response time was 9 minutes for swelling and 14 minutes for deswelling. The average magnitude response change was 2.4 KPa for swelling and 2.6 KPa for deswelling.

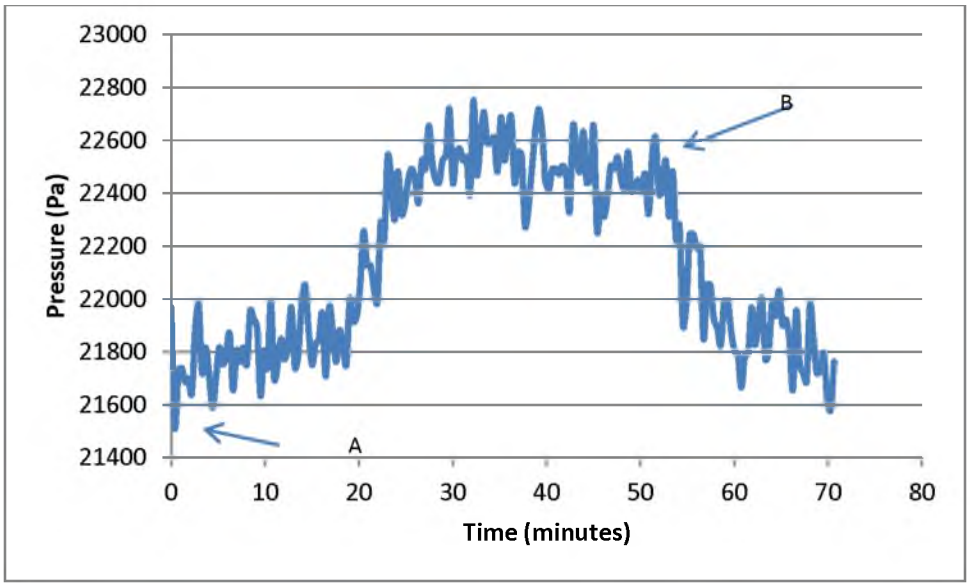

Figure 5.3. An ionic strength test after 18 months where the average first order response time was 9 minutes for swelling and 8 minutes for deswelling. The average magnitude response change was 1.1 KPa for swelling and 1.6 KPa for deswelling.

samples swell at low ionic strength concentrations and swell as the ionic strength concentration increases.

The data represented in these graphs illustrate that the hydrogel has the ability to respond to changes in ionic strength. Furthermore, they illustrate that the hydrogel continues to be responsive after being stored in a stock solution of PBS for extended periods of time.

5.3.2 Transportation Testing

The control hydrogel sample was tested under the conditions outlined above. The average response time for swelling was 74 hours with a magnitude of 6.2 KPa. The average deswelling response time was 45 hours with a magnitude of 5.6 KPa (see Figure 5.4).

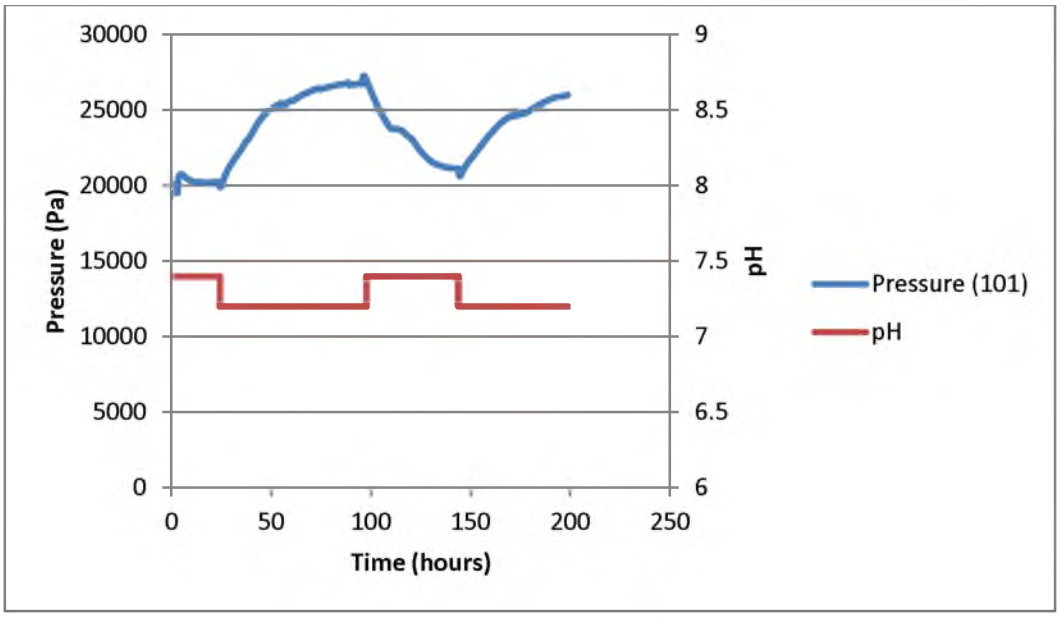

Figure 5.4. The control test of the chemomechanical sensor showing the pH response from 7.2 to 7.4 prior to simulated transportation

The same test was performed on another hydrogel sample of identical composition in the same chemomechanical sensor after simulated transportation (see Figure 5.5). The average response time for swelling in this experiment was 35 hours with a magnitude of 19 KPa. The average deswelling response time was 28 hours with a magnitude of 17 KPa.

5.3.3 Operational Stability

The initial test was conducted within 1 week of synthesizing the hydrogel. The primary objective of this test was to determine the sensitivity of this hydrogel to small changes in ionic strength concentrations. The data show that the swelling magnitude is smaller than the deswelling magnitude (see Figure 5.6).

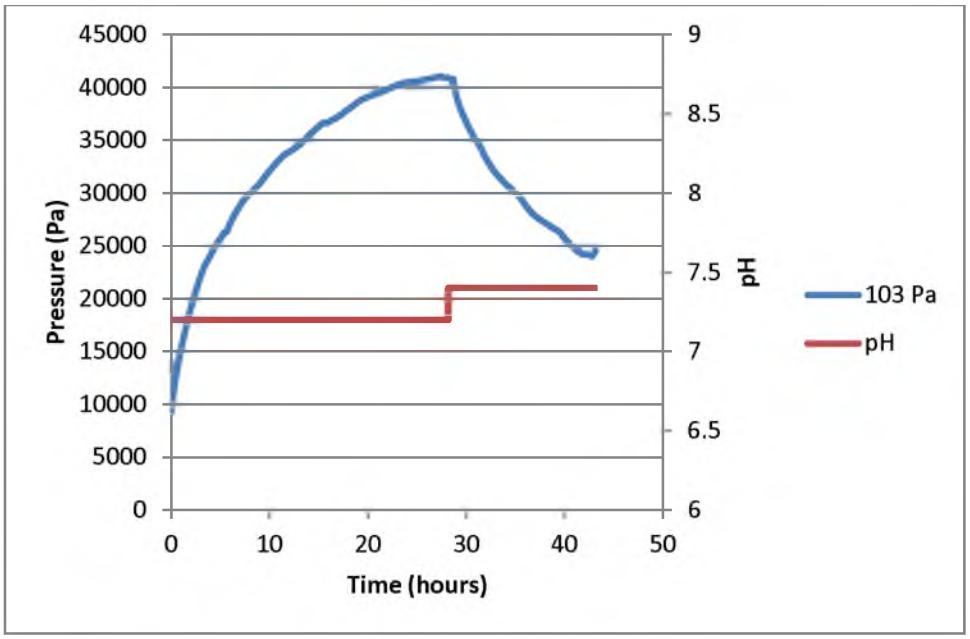

Figure 5.5. A pH response test from pH 7.2 to 7.4 of the hydrogel sample in the M-Biotech sensor after simulating travel conditions

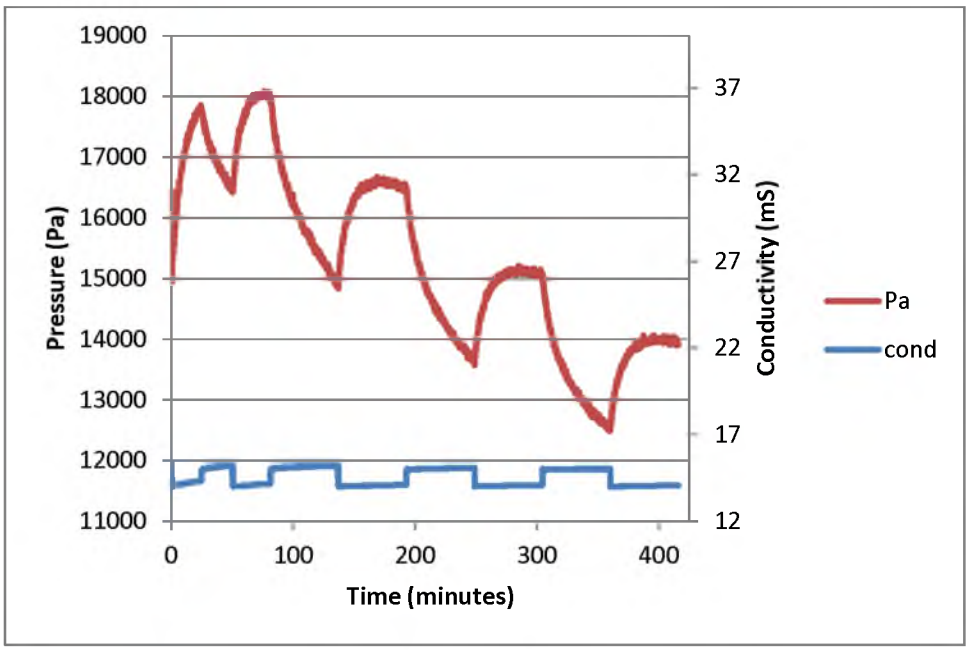

Figure 5.6. An ionic strength test immediately after synthesis where the initial test was conducted within 1 week of the hydrogel synthesis. The hydrogel was tested for 5 continuous cycles. After the first 2 cycles, the continuous system was modified in order to allow the swelling and deswelling to come to an equilibrium point.

The second test was performed after 9 months of storage. The objective of this test was to determine if the magnitude of the response to the change in ionic strength concentration would change after repeated testing (see Figure 5.7). The response magnitude and time were taken at different time intervals to determine the change (see Table 5.1).

Based on these data, the magnitude of the deswelling response was greater than that of the swelling response for the first 7000 minutes (40 cycles). In addition, the deswelling response time decreases as the number of cycles increases. As the swelling and deswelling response approaches equilibrium, there was no significant difference between the response times and magnitudes in either swelling or deswelling. However, there remained a difference between the swelling and deswelling response times, which was also noted in the test after one week of synthesis. After 40 cycles, the response of the hydrogel reached equilibrium, where the magnitude of the response for swelling was equal to the magnitude of the response for deswelling.

A sample of the hydrogel was tested again at 18 months to determine the response times and magnitudes at different time intervals. The objective of this test was also to determine if the response time and magnitude would change with multiple cycles. As the test at 9 months yielded no significant change as it approached equilibrium, it was decided to test this hydrogel with 100 cycles (see Figure 5.8). As with the test at 9 months, the response time and magnitude data were collected at set time intervals (see Table 5.2). This test showed that the hydrogel was tested through 25 cycles before the hydrogel was able to reach equilibrium. After the initial 25 cycles (1600 minutes) the hydrogel obtained stability, and the response times and magnitudes remained constant.

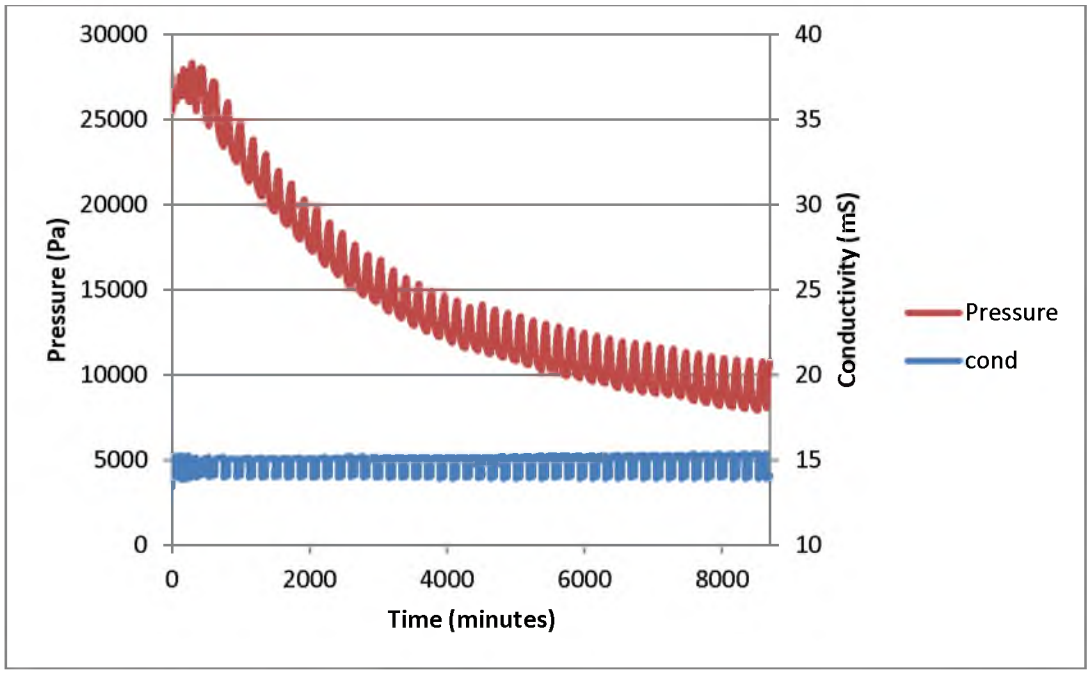

Figure 5.7. An ionic strength test after 9 months where the hydrogel was tested for 51 continuous cycles.

Table 5.1. The swelling and shrinking response times and magnitudes at various time intervals illustrate the stable response of the hydrogel.

Swelling			Shrinking		
Time (minutes)	Response Time (minutes)	Magnitude (KPa)	Time (minutes)	Response Time (minutes)	Magnitude (KPa)
1000	9	2.37	1000	19	3.35
3000	11	2.43	3000	19	2.77
5000	9	2.4	5000	14	2.63
7000	9	2.7	7000	15	2.85
9000	9	2.55	9000	13	2.75

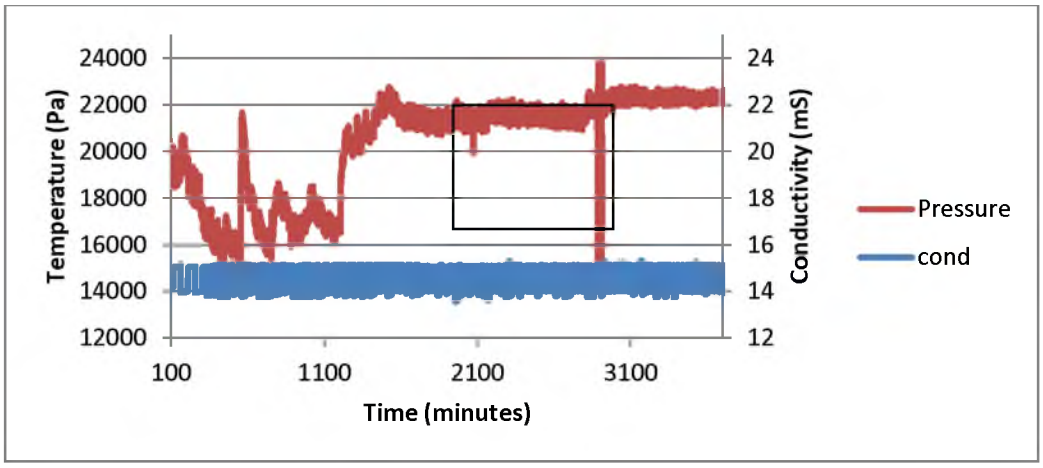

Figure 5.8. An ionic strength test after 18 months of synthesis where the hydrogel was tested for 100 continuous cycles. The portion of the graph within the box is magnified in Figure 5.9.

Table 5.2. The swelling and shrinking response times and magnitudes at various time intervals illustrate the stable response of the hydrogel.

Swelling			Shrinking		
Time (minutes)	Response Time (minutes)	Magnitude (Kpa)	Time (minutes)	Response Time (minutes)	Magnitude (Kpa)
700	4	1.128	700	6	0.935
1400	6	1.085	1400	8	0.958
2100	6	0.989	2100	7	0.947
2800	5	0.912	2800	6	0.955
3500	7	0.977	3500	7	0.955
4000	6	0.904	4000	7	0.911

A follow up test was performed with the same hydrogel sample in a different pressure sensor because the sample lost a small amount of sensitivity at the end of the initial 100 cycle test. This test was used to determine whether the loss of magnitude of the response was due a change in the hydrogel or in the sensor. The results of this test show that the average magnitude response of this test is 1.4 KPa for swelling with a response time of 4 minutes. The average deswelling magnitude is 1.2 KPa with a

response time of 3 minutes. Furthermore, there was no significant difference between the magnitude of the response at the beginning of the test and the response at the end of the test. This validation experiment confirmed that the irregular response of the first test at 18 months was due to the sensor and not the hydrogel sample.

5.4 Discussion

The results indicate that the hydrogel samples are responsive to ionic changes, even after an extended period of time in storage. The data gathered from the first two experiments show only a negligible amount of noise, while the third experiment shows a much higher signal to noise ratio. The same piezoresistive sensor was used in all three experiments. As time progresses, the piezoresistive sensing diaphragm loses stability, which generated the noise during the third experiment and likely the decreased response of the hydrogel.

The hydrogel was conditioned for 3 cycles from 55 mM to 165 mM of PBS. The purpose of the conditioning is to create a controlled environment for the hydrogel to swell and deswell. However, the number of cycles was only arbitrarily chosen. The deswelling response of the hydrogel from the first test continued to have a higher magnitude than the swelling response. In addition, the second and third tests both reveal that this magnitude difference can be overcome and equilibrium can be reached after approximately 25 – 30 cycles. The third test demonstrated the most promising results, illustrating that the hydrogel can be tested for more than 40 cycles with consistent magnitudes of swelling and deswelling; however, this only occurred after the initial 25 –

30 cycles. The stable region of the 100 cycle test after 18 months is magnified in Figure 5.9.

5.4.1 Storage Stability

After synthesis, the hydrogel monolith was stored at room temperature in PBS solution. The solution was not changed and the hydrogel was stored in natural ambient light. Samples taken from the monolith were within 1 mm of the previous sample taken. This was done in order to obtain results from a homogeneous sample. The data gathered from each test indicate that the hydrogel is able to respond after extended periods of time in storage. This suggests that the shelf life under ambient conditions is greater than 18 months. A figure comparing the response times and magnitudes at the different time intervals is given in Figure 5.10.

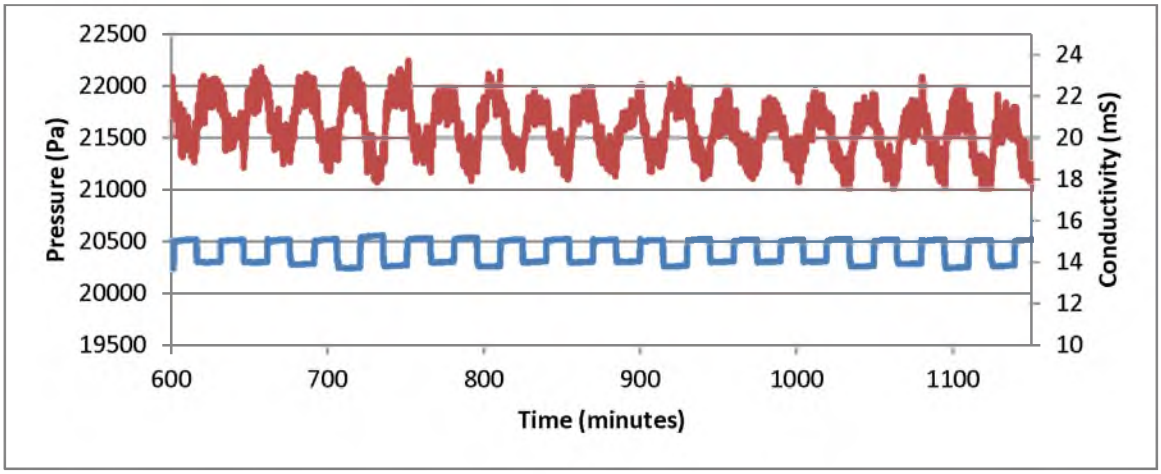

Figure 5.9. A stable region of the 300 cycle test shows 18 cycles of the third test that illustrate the stability of the hydrogel swelling and deswelling.

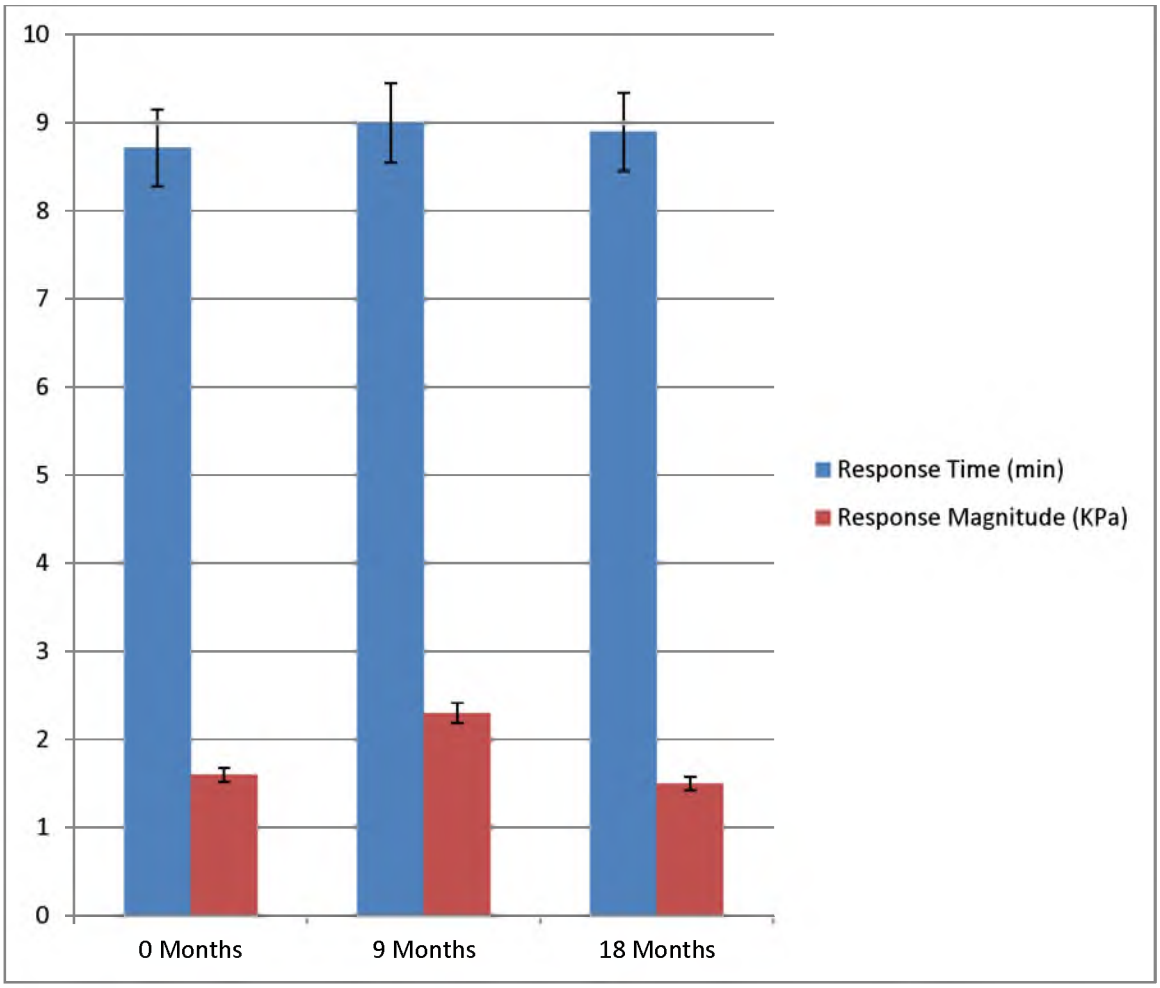

Figure 5.10. A comparison of the response stability

5.4.2 Transportation Tests

The hydrogel samples used in this project were all of the same composition. As discussed in Reference 12, the composition was designed so that the hydrogel swells at low pH. The composition used in this project was designed to have a high sensitivity, and therefore has a higher concentration of DMA than compositions used in other studies [12]. The experiment for the transportation test was designed to determine the effect of response time and magnitude on chemomechanical sensors after experiencing vibrations

and exposure to uncontrolled temperatures. The simulated conditions provided evidence that the sensor could be used for further experimentation.

The sensor data gathered from the simulated transportation experiment show that the hydrogel maintains a response to changes in environmental stimuli after transportation, though changes do occur. The data from the control experiment demonstrate a lower magnitude response, 6 KPa compared to 19 KPa after the transportation test, and a longer response time, 75 hours compared to 35 hours. When the hydrogel monolith is synthesized, there are differences in the optical properties across the monolith. The synthesized monolithic hydrogel was not homogenous, and the cross-link density of the hydrogel decreased after simulated transportation. Variation in UV intensity during photocross-linking may also have had an effect. While the two experiments differ in their results, the data demonstrate that the hydrogel maintains its response to changes in environmental stimuli.

5.4.2 Operational Stability

There are several factors that may influence the response time and magnitude of a hydrogel sample. The data gathered during the four experiments demonstrate that the hydrogel has the ability to respond to continuous cycles. The magnitude and time of the responses during each of the tests varied slightly from test to test, but the group of tests shows that the hydrogel will maintain a measurable response to repeated testing. The magnitude and time of the response for the last 20 cycles of the 100 cycles test began to decrease. In order to determine if this was a loss of mechanical properties, a second test was performed on the same hydrogel sample used in that test. The hydrogel was tested in

a different sensor for an additional series of cycles to determine the response of the hydrogel after that time. The response time and magnitude remained constant through the follow-up test. The data gathered in the second experiment have helped determine that the decrease in the response was due to a problem with the sensor rather than a loss of response due to the swelling and deswelling behavior of the hydrogel.

5.5 Conclusions

The experiments performed in this project were designed to determine if a hydrogel sample could be stored for an extended period of time and to determine if a hydrogel sample could be tested continuously. Samples taken from a hydrogel monolith were tested immediately after synthesis and after 9 and 18 months of storage at ambient conditions. The hydrogel responded in the same manner for all three of the tests. The experimental results obtained in this project demonstrate that hydrogels can be synthesized, dried, and then rehydrated after a period of time without losing their ability to respond to environmental stimuli. The hydrogel samples were also tested continuously through repeated cycles to determine the effects of the hydrogel after prolonged testing. The hydrogel responded with a similar magnitude and response time throughout the continuous testing with no significant decrease in sensitivity. The results of these tests demonstrate that hydrogels can be used after being stored for an extended period of time, can withstand the stresses of shipping and can be used in continuous cycle testing.

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CHAPTER 6

AN IMPROVED DESIGN FOR CHEMOMECHANICAL SENSORS: A PIEZORESISTIVE PRESSURE SENSOR WITH A MECHANICAL BOSS

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J. Bates, P. Tathireddy, S. Buetefisch, J. Magda, An Improved Design for Chemomechanical Sensors: A Piezoresistive Pressure Sensor with a Mechanical Boss, Chemosensors (2013) 33-42

6.1 Introduction

Hydrogels are super absorbent network polymers consisting of three-dimensional structures that can absorb and retain water and other aqueous fluids while maintaining the original structure. Hydrogels are made of water soluble monomer backbone molecules with a cross-linking molecule selected for either physical or chemical properties. Hydrogels are good candidates in biomedical applications because of their response to changes in the local environment. Hydrogels may swell or deswell depending on the conditions of the surrounding aqueous media. The swelling response is currently being harnessed in biological sensing applications for the detection of both analytes in solutions and biological compounds [1-8]. Hydrogels are known to respond to changes in pH, glucose concentration, ionic strength, temperature, electric field, solvent composition,

and pressure. The swelling response of pH-responsive hydrogels occurs as nitrogen groups on the dimethylaminoethyl methacrylate (DMA) molecule become positively charged, which causes an electrostatic repulsion between neighboring DMA molecules.

In a chemomechanical sensor, this hydrogel swelling response is transduced into a measureable signal when the hydrogel exerts a stress on the diaphragm of a miniature pressure transducer. While chemomechanical sensors have been used with promising results in a number of different studies [9-24], improvements in design are still needed in order to improve sensor robustness and response kinetics without sacrifice of sensitivity.

There are several research groups who have worked on hydrogel-based sensors that measure the pressure exerted by a swelling response. The results of some of these projects are shown in Table 6.1.

Table 6.1. A comparison of pH-responsive hydrogel results from reference projects with the composition, hydrogel thickness, and response time of each project

Hydrogel Backbone/Sensing Group	Hydrogel Thickness	pH Response Time	Reference
Hydroxypropyl Methacrylate/ Dimethylaminoethyl Methacrylate	50 μm	15 minutes	9-11
Polyvinyl Alcohol/Poly acrylic Acid	50 μm	6 minutes	14
Acrylamide/Phenyl Boronic Acid	400 μm		16
Hydroxypropyl Methacrylate/ Dimethylaminoethyl Methacrylate	400 μm	135 minutes	17-20
Polyvinyl Alcohol/Poly acrylic Acid	50 μm	30 minutes	22
Polyvinyl Alcohol/Poly acrylic Acid	40 μm	78 minutes	23

The data presented in Table 6.1 compare the hydrogel composition, thickness, and response time across several research projects. Not all of the research projects provided results for the composition, thickness, or response time in their projects because those parameters were not the main focus of their research. Furthermore, the focus of the research projects listed in Figure 6.1 is not the same as the research presented here. Some groups measure the swelling from dehydrated to a hydrated state [22], while others address the diffusion and swelling kinetics to characterize the swelling response [14]. These projects are provided here to illustrate the inconsistency of these parameters among research projects. One sensor assembly, referred to as the Han et al. sensor [16], utilizes cylindrical samples of a hydrogel and a macro piezoresistive sensor to measure changes in the swelling pressure. Another group [23] created a sensor assembly with an elevated platform. This platform makes it possible to test hydrogel samples with a decreased thickness. This sensor utilizes microchannels for fluid exchange. The pedestal design was further investigated in this project, and is referred to as the piezoresistive pressure sensor with a mechanical Boss, or the Boss sensor.

In this study, two different chemomechanical sensor designs are compared. In the first and older design, which dates back at least to 2002 [16], the hydrogel is synthesized in a mold and then placed into the sensor using a screw-on cap with a porous membrane top. This sensor design is referred to as the Han sensor [16]. This simple design has been successfully used in a number of studies [12-19], but is not well suited for use with thin hydrogels because of excessive compliance within the chamber that confines the hydrogel against the pressure transducer diaphragm [12]. In the second and newer design, the same type of hydrogel is synthesized *in situ* between a porous membrane and a boss,

a protruding component on the sensor, which is mechanically connected to the pressure transducer diaphragm. This design is more difficult to fabricate but allows thinner hydrogels to be used, which should lead to smaller sensor response times. The objective of this study is to compare the response times and magnitudes of the two chemomechanical sensors using the same pH-responsive hydrogel, and to determine the advantages and disadvantages of each sensor.

6.2 Experimental Methods

6.2.1 Materials

The following monomers were used as received from Sigma Aldrich: 2-hydroxypropyl methacrylate (HPMA), dimethylaminoethyl methacrylate (DMA), and tetraethylene glycol dimethacrylate (TEGDMA). In addition, 2,2-dimethoxy-2-phenylacetophenone (DMPAP), a photoinitiator, and ethylene glycol (EG), a solvent for the pregel solution, were also obtained from Sigma Aldrich and used as received. Dulbecco's phosphate buffered saline (PBS) was mixed at 9.6 g/L in deionized water, and (3-aminopropyl) triethoxysilane (APTES) was prepared as 5% in solution with ethanol. Both the PBS and APTES were obtained from Sigma Aldrich.

6.2.2 Hydrogel Monolith Synthesis

Hydrogel monoliths were synthesized in a mole ratio of 76.1 DMA, 2.2 HPMA, 0.6 TEGDMA, and 21.1 EG. The pregel solution was purged with argon gas, and injected into a synthesis module creating a monolith with a thickness of 400 μm. The synthesis module was also purged with argon gas and consisted of two glass plates with a 400 μm

spacer. The injected pregel solution was exposed to ultraviolet light at 365 nm for 90 seconds, which activated the photo initiator and resulted in free radical polymerization. After polymerization, the hydrogel monolith was removed from the synthesis module, washed with deionized water, and stored in PBS for 24 hours prior to conditioning. The hydrogel was conditioned by alternating ionic strength conditions every 4 hours for at least 3 cycles. This allows the hydrogel to swell and deswell to remove unreacted monomers from the hydrogel matrix.

6.2.3 Han Chemomechanical Sensor Design Specifications

The swelling pressure of the hydrogel samples was measured using two different chemomechanical sensors. The first, the Han sensor, consisted of a piezoresistive sensor (EPX Series, Measurement Specialties, Hampton, Virginia, USA) and a cap containing a porous mesh membrane (see Figure 6.1). This device encloses the piezoresistive sensor and the hydrogel, while allowing fluid exchange between the exposed surface of the hydrogel and the surrounding environment. The cylindrical sample of hydrogel (3.5 mm diameter and 400 μm height) was placed in the chemomechanical sensor and held in place using the screw-on cap. The porous membrane was a stainless steel wire cloth mesh (120) from Small Parts, Inc., Logansport, Indiana, USA. The sensor was placed in a stirred temperature controlled bath, and the signal was transmitted to a PC using an Agilent 34970A Data Acquisition Device (Santa Clara, California, USA).

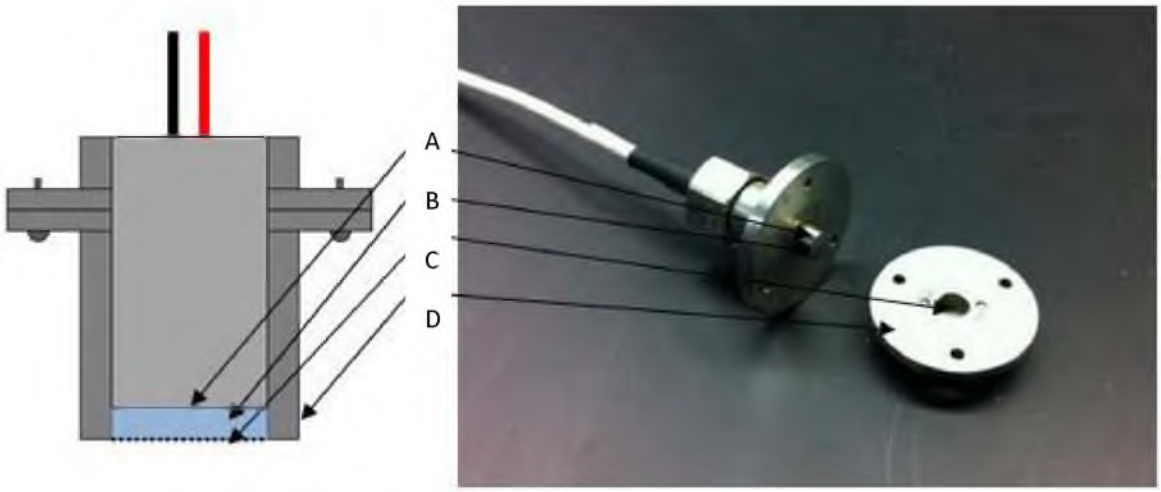

Figure 6.1. The set up for the Han chemomechanical sensor includes a piezoresistive surface pressure sensing surface (A), a hydrogel sample of 400 um in thickness and 3.5 mm in diameter placed on the sensor (B), a stainless steel sensor cap (C), and a mesh membrane to allow fluid exchange (D).

6.2.3.1 Converting the Signal

The signal output of both sensors was collected in units of mV. The sensitivity of the pressure sensor was characterized with a water column, and the signal was converted to units of Pascals with equation 6.1.

$$P = SV + X \tag{6.1}$$

In this equation, P is the pressure calculated, S is the sensitivity of the sensor used, V is the voltage obtained from the pressure sensor, and X is a scaling parameter based on the baseline data of the sensor obtained prior to sample testing.

6.2.4 Boss Chemomechanical Sensor-Design Specifications

A new chemomechanical sensor design was used with the miniaturized hydrogel. The sensor consisted of a silicon pressure diaphragm and a mesh cover. The surface of

the pressure sensor was 1 mm^2 with a distance between the boss and a porous mesh of 50 µm. Therefore, utilizing this pressure sensor limited the thickness of the hydrogel sample to 50 µm (see Figure 6.2).

The swelling stress of the hydrogel is transmitted to the piezoresistive diaphragm via a silicon boss. The porous mesh was the same stainless steel wire used in the Han chemomechanical sensor design (see above). Data from the Boss sensor were also collected in units of voltage.

The response time for both sensors was determined as the duration of time from the initial change in the environmental conditions to the time where the hydrogel response came to equilibrium and maintained a stable response (+/- 0.1 mV).

6.2.5 Surface Preparation of Boss Sensor

The silicon surface of the boss was hydrophilic; therefore, surface preparation with 5% APTES in ethanol was used to increase adhesion of the hydrogel pregel solution

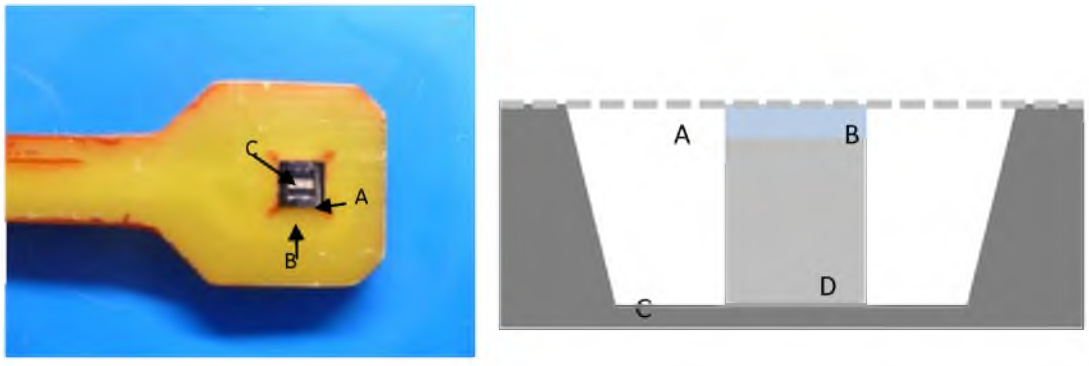

Figure 6.2. The image on the left shows the actual microsensor assembly. The image on the right shows a cross sectional illustration of the sensor assembly. In the image (A) is the mesh membrane, (B) is the hydrogel sample, (C) is the piezoresistive diaphragm, and (D) is the boss.

to the silicon surface. A silicon wafer was used to simulate adhesion promotion and surface treatment prior to treating the sensor. One silicon wafer was treated with APTES and the other was controlled and left unchanged. The silicon wafer was treated with 5% APTES by dipping the wafer in the solution five times and then immediately removing the residue in deionized water. The solvent was removed from the surface by low pressure argon blowing. A small amount of hydrogel pregel solution was synthesized with UV exposure on the surface of both treated and untreated silicon wafers, and adhesion was increased on the treated surface. After testing the experimental procedure on a silicon wafer, and determining that the adhesion was increased on the silicon surface, the surface of the pressure sensor was treated with APTES by following the same procedure.

6.2.6 *In Situ* Synthesis

The pressure sensor was preassembled, and the hydrogel was injected through the mesh membrane. The hydrogel was synthesized in a glove box in an inert environment. The sensor was placed under a microscope and 1.5 μL of the pregel solution was placed on the surface of the mesh membrane. The pregel solution was observed until it had passed through the mesh membrane. UV light with a wavelength of 365 nm was immediately exposed to the pregel solution until polymerization was complete. The hydrogel was not hydrated until testing began.

6.2.7 Testing Procedures

6.2.7.1 Experiment 1: pH Response of 50 μm Hydrogel Using Boss Sensor Design

The boss sensors were loaded with a hydrogel sample and tested from pH 4.0 to 7.0 in 1X PBS at a constant ionic strength of 165 mM and temperature of 25 ^{o}C to determine the pH response time and magnitude. A cross-sectional illustration of the microsensor is shown in Figure 6.2.

6.2.7.2 Experiment 2: pH Response of 400 μm Hydrogel Using Han Sensor Design

A 3.5 mm diameter sample was removed from the hydrogel monolith using a biopsy tool and loaded into the Han sensor of Figure 6.1. The hydrogel was tested from pH 4.0 to 7.0 in 1X PBS at a fixed ionic strength of 165 mM and at temperature of 25 ^{o}C to determine the pH response time and magnitude for comparison to the Boss sensor.

6.3 Results

6.3.1 Experiment 1: pH Response of 50 μm Hydrogel in Boss Sensor Design

A test was performed on the miniaturized hydrogel in the Boss design sensor (see Figure 6.3). The average swelling response time was 0.34 hours, with a magnitude of 2.04 KPa. The average deswelling response time was 0.07 hours, with a magnitude of 2.15 KPa.

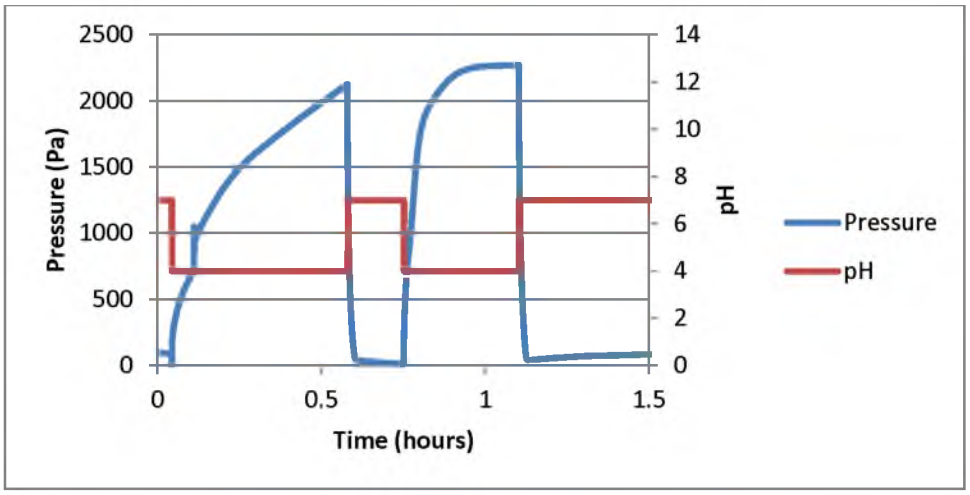

Figure 6.3. Representative data showing 2 cycles of the pH response from the Boss sensor from pH 4.0 to 7.0

6.3.2 Experiment 2: pH Response of 400 µm Hydrogel in Han Design Sensor

A test was performed on a sample of the 400 µm hydrogel sample in the Han sensor under the same pH change as the Boss sensor design (see Figure 6.4). The average swelling response time was 20 hours with a magnitude of 1.63 KPa. The average deswelling response time was 30 hours, with a magnitude response of 1.22 KPa.

6.4 Discussion

The data given in Figure 6.5 compare the response time and magnitude of the boss sensor and the Han sensor for a pH change of 4.0 to 7.0. The pH response of the boss sensor loaded with the 50 µm hydrogel had a faster response time, 0.34 hours compared to 20 hours for the 400 µm hydrogel. However, the response magnitude remained somewhat constant for the 50 µm hydrogel, 2.04 KPa compared to 1.63 KPa for the 400

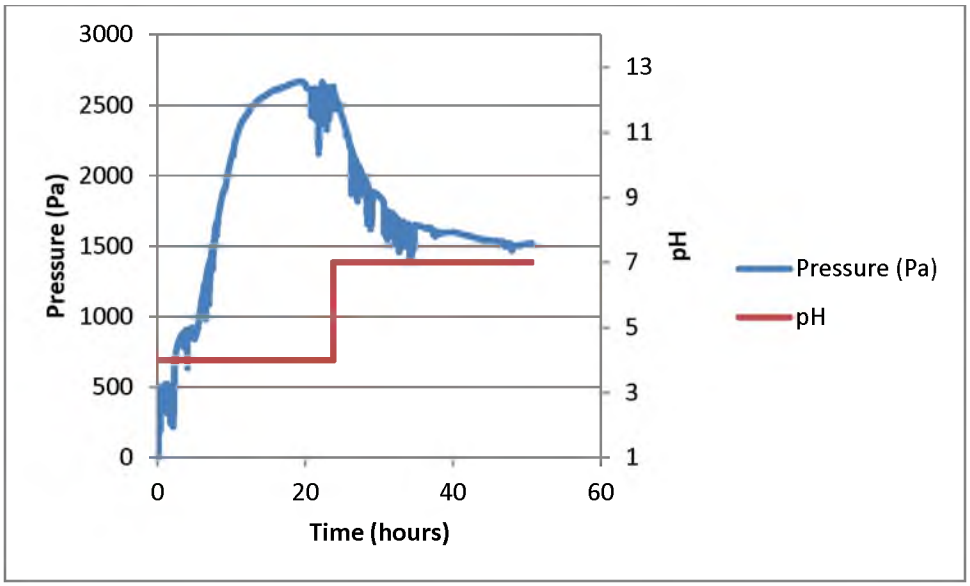

Figure 6.4. The representative pH response from pH 4.0 to 7.0 of the 400 μm hydrogel in the Han sensor

μm hydrogel. Hence, the new boss sensor design successfully reduces the response time without sacrificing response magnitude. The hydrogel thickness reduction is the factor responsible for the reduced response time of the boss sensor, which will likely have decreased the diffusion time through the hydrogel matrix. Assuming the sensor response is under diffusion control, the response time should be $8^2 = 64$ times smaller for the boss sensor. The observed difference is 60 times smaller.

The Han design provides advantages that include convenient reuse with different hydrogel samples, and the ability to use the sensor with hydrogel samples of uniform thickness. In addition, this design allows the researcher to load the sample with a pre-determined pressure value by adjustment of the screw-on cap prior to experimentation. On the other hand, the Boss sensor can be used to test thinner hydrogel samples, which results in a faster response time. In addition, the sensor can be easily miniaturized for use in biomedical monitoring.

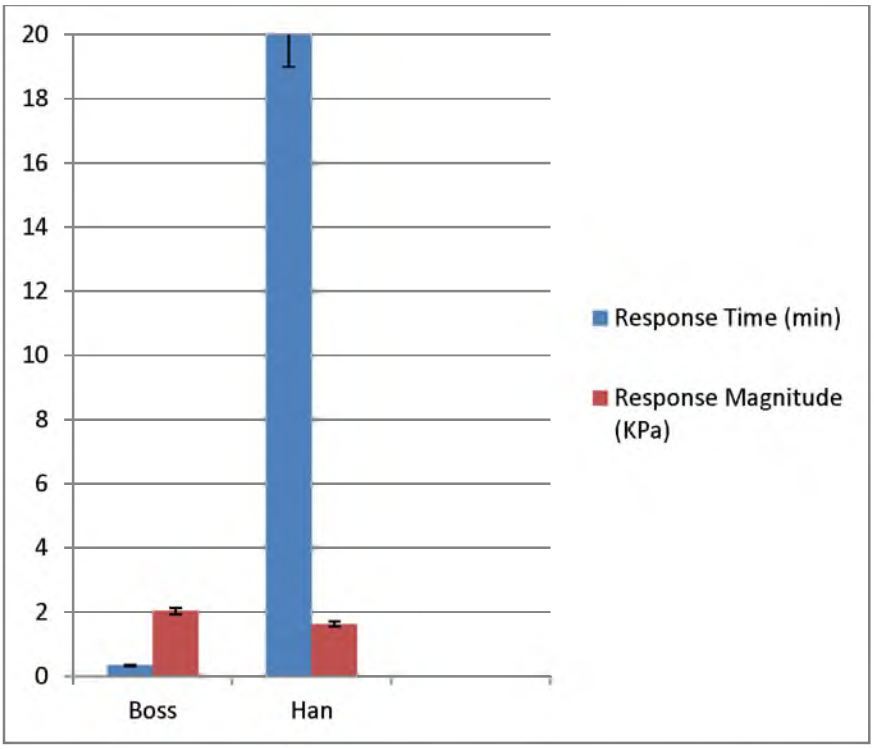

Figure 6.5. A comparison of response times and magnitudes of the experiments performed

6.5 Conclusion

Comparison has been made between two different designs of chemomechanical sensors: the Han design and the Boss design. The Han design is more convenient for reuse with different hydrogel samples. However, the Boss sensor can be used with thinner hydrogel samples to give faster sensor response kinetics without loss of sensitivity. When the Boss sensor is compared to the other sensors presented in Table 6.1, the Boss sensor has a simpler design that does not require the use of sampling of media solution, nor does it employ the use of microfluidic channels. Therefore, the Boss sensor provides a useful application for continuous monitoring of biological systems.

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CHAPTER 7

FABRICATION OF A CHEMOMECHANICAL SENSOR USING 3D PRINTING TECHNOLOGY

7.1 Introduction

The use of piezoresistive pressure sensors has been proven as an effective signal transduction method for measuring the hydrogel swelling response [1-14]. The major limitations to this technology are the high cost and the long production time of the piezoresistive sensors that are commercially available. In this chapter, an inexpensive off the shelf pressure sensor was used in combination with 3D printed parts to address the high cost and long production time [15].

7.2 Materials and Methods

7.2.1 Hydrogel Synthesis

Hydrogel monoliths responsive to pH were synthesized by copolymerizing the monomers dimethylaminoethyl methacrylate (DMA), 2-hydroxyethyl methacrylate (HEMA), and tetraethylene glycol dimethacrylate (TEGDMA) in the nominal mole ratio of 86.1:2.1:0.3. This is the optimized composition described in Chapter 4. The monomers were mixed with a photoinitiator (2,2-dimethoxy-2-phenylacetophenone) in the solvent

ethylene glycol, purged with argon gas, and then injected between two glass plates separated by a teflon spacer. Free radical cross-linking copolymerization was initiated by UV irradiation for 90 seconds (365 nm). The monoliths were conditioned prior to testing and loaded into a chemomechanical sensor.

7.2.2 Signal Transduction

A 3D printed sensor cover was used in combination with a piezoresistive sensing diaphragm. This new design was created to decrease the cost and time associated with the macrosensor used in other experiments. The macrosensor design utilized an expensive pressure sensor and the sensor cap was machined of stainless steel in a machine shop that was both expensive and time consuming. The 3D printed sensor utilized a sensor cover designed with SolidWorks software (Dassault Systems SolidWorks Corporation, Waltham, MS, USA); components printed with Objet VeroWhitePlus, a methyl methacrylate polymer, on an Objet 3D printer (Stratasys, Eden Prarie, MN, USA); and a piezoresistive pressure sensor obtained from AktivSensor (EPCOS AktivSensor GmbH, C41 Series, Stahnsdorf, Germany). The voltage measurement was used to determine both the response time and magnitude. The response time was calculated as the time from the initial change in environmental pH to the time at which the hydrogel swelling pressure response reached a stable value (+/- 0.1 mV).

7.2.3 Testing Procedures

The 3D printed material was placed in water for 24 hours to determine water permeability of the 3D printed polymer. The thickness and width of the sensor cover

were measured with a micrometer. The weight was also measured with a balance. The measurements are shown in the results section.

The swelling pressure of the hydrogel in the chemomechanical sensor was measured using a piezoresistive pressure sensor with a cap containing a porous mesh membrane [4-5]. This device enclosed the piezoresistive diaphragm and the hydrogel, while allowing the hydrogel to interact with the fluid of the surrounding environment. A cylindrical sample of hydrogel, 3.5 mm in diameter and thickness between 400 μm, was inserted in the chemomechanical sensor, which was then placed into the stirred testing chamber. Sensor response tests were performed at a fixed ionic strength of 165 mM and at a fixed temperature of 25 ^{o}C, while the pH of the media solution was changed from to 7.4 to 7.2.

7.3 Results

7.3.1 3D Printed Sensor

The 3D printed sensor cover was designed on Solid Works. A schematic representation of the printed parts is shown in Figure 7.1. An actual image of the parts is shown in Figure 7.2. The AktivSensor was wire bonded based on the diagram shown in Figure 7.3. The wire bonded sensor was placed into the 3D printed sensor, and is shown in Figure 7.4.

7.3.2 Swelling Data from the 3D Printed Material

The sensor cover was placed in water and measurements were collected every hour for 24 hours. The results are given in Table 7.1. As shown from the results, the

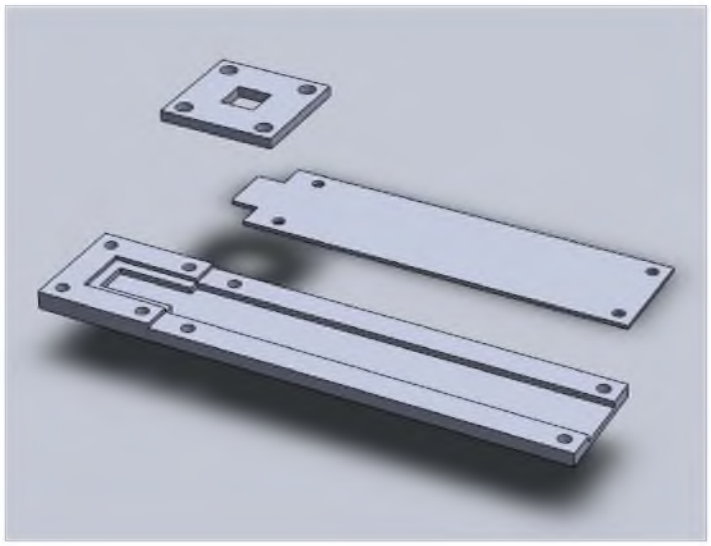

Figure 7.1. SolidWorks drawings of the components of the 3D printed piece. The pressure sensor is placed at point A, and a void present to ensure the sensing diaphragm is able to bend.

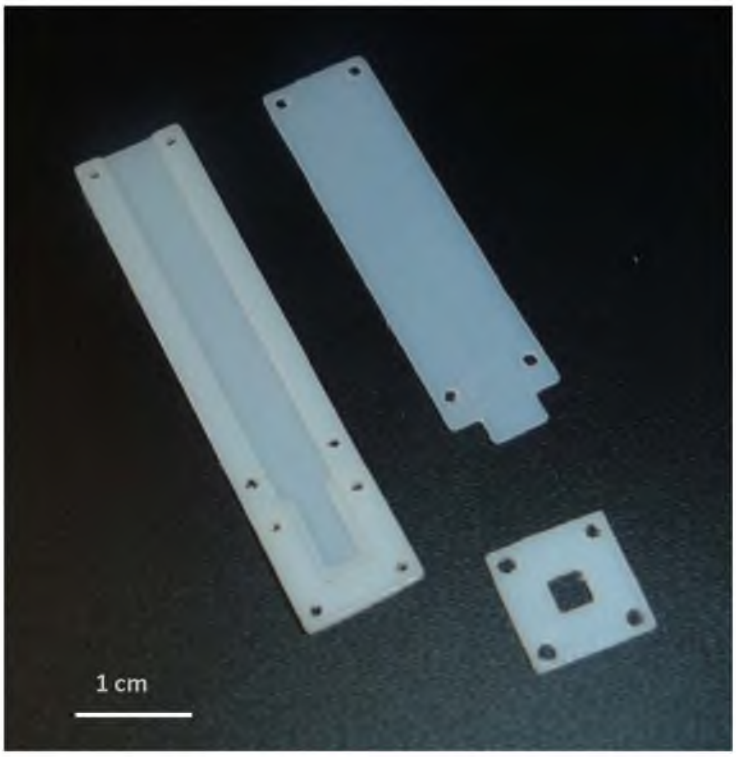

Figure 7.2. An image of the actual 3D printed parts.

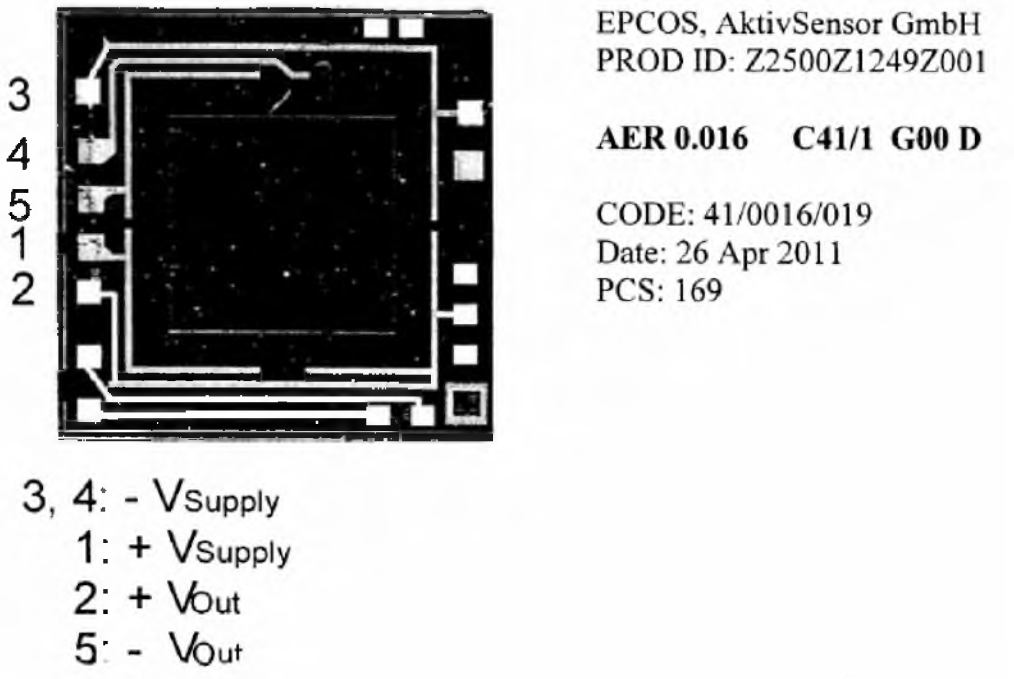

Figure 7.3. A schematic diagram used during the wire bonding process

Figure 7.4. The assembled sensor with a loaded hydrogel sample directly under the mesh membrane shown on the left.

Table 7.1. The swelling data from the 3D printed sensor cover shows that there is negligible absorption of water.

Time (hours)	Height (mm)	Width (mm)	Weight (g)
1	0.46	3.86	0.0515
2	0.46	3.86	0.0515
3	0.46	3.86	0.0515
4	0.46	3.86	0.0515
5	0.46	3.86	0.0515
6	0.46	3.86	0.0515
24	0.46	3.86	0.0515

measurements of the sensor cover did not change in a 24-hour time period; therefore, the 3D printing material does not absorb a measurable quantity of water.

7.3.3 Preliminary Data from the 3D Printed Sensor

After the 3D printed sensor was assembled, the sensing diaphragm was connected to a power supply, and a graph of the time versus signal output was generated. Small droplets of water (1 μL) were placed on the sensing diaphragm to detect small changes in the signal output (Figure 7.5). The signal output generated some noise, but the fluctuations in the electrical signal are seen at three distinct points on the graph.

7.3.4 pH-Responsive Test from the 3D Printed Sensor

The sensor was assembled with a sensor cover, a mesh membrane, a hydrogel sample measuring 400 μm, and the piezoresistive sensor diaphragm. The sensor was placed into a solution with a pH of 7.2, and allowed to swell. The voltage data are given in Figure 7.6.

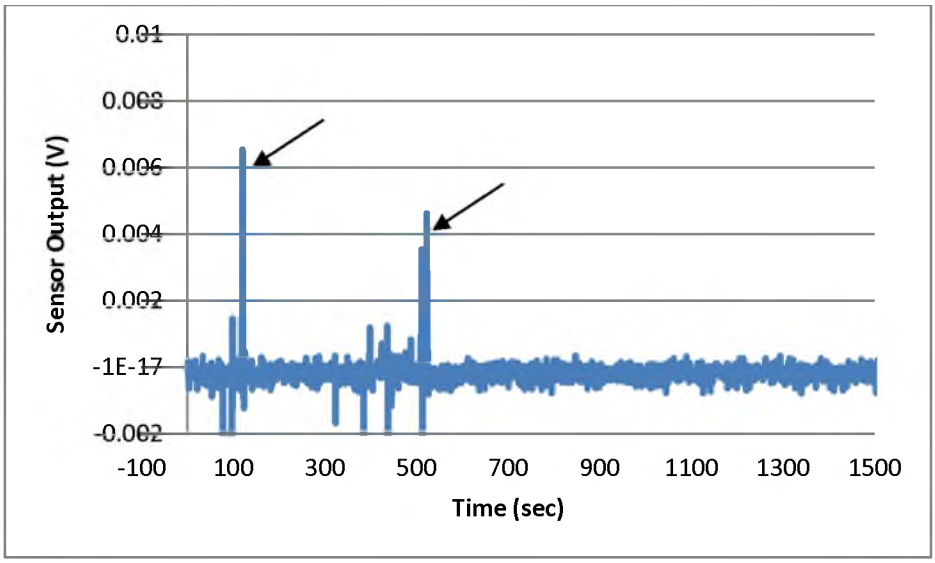

Figure 7.5. A plot of the time vs. sensor output. The spikes in the signal (as indicated by the arrows) represent an increased load on the sensing diaphragm, where water droplets were placed on sensor.

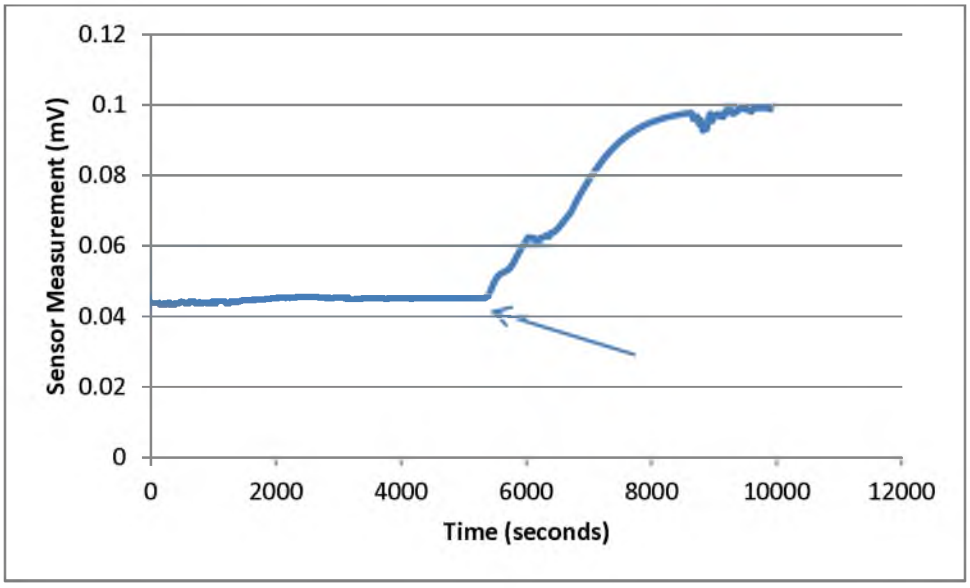

Figure 7.6. A pressure test demonstrating the change in swelling pressure at a pH of 7.2. The arrow shows the point in time where the conditions were changed.

The magnitude of the hydrogel response (67 KPa) was larger than the pressure response threshold of the sensing diaphragm (60 KPa), which result in the cracking of the pressure sensor.

7.4 Discussion

7.4.1 3D Printed Material Considerations

The swelling test of the 3D printed sensor cover demonstrates that the sensor does not appear to absorb water, which indicates that the material could be suitable for 3D printed sensor arrays. The 3D printed sensor cover was originally designed to be used with screws, which would enable reusability of the sensor and the ability to change hydrogel samples. However, during prototyping, it was noted that the mechanical properties of the 3D printing material were not ideal for use with screws, therefore limiting the reusability of the sensor. The modulus of the VeroWhite Plus polymer is calculated using equation 7.1, which considers the thickness of the material. The two thicknesses were 0.5 mm and 1.0 mm.

$$E = \frac{\sigma^2 wht}{2W} \tag{7.1}$$

In equation 7.1, W represents the elastic strain energy of a plate with no crack, w represents the width, h represents the height, t represents the thickness, and E represents the Young's Modulus. The resulting Young's modulus for 0.5 mm thickness is 45 MPa. This low value, which represents the thickness of the material in the first prototype, is responsible for the inability to capture a pressure response. The thickness of the 3D

printed material was increased to 1 mm, which gave a Young's modulus of 5880 MPa. The 1 mm thickness was used to capture the signal demonstrated in Figure 7.6.

Because of limitations with regulating the loading pressure, the sensor was assembled with an acrylate based polymer adhesive. The adhesive increased the mechanical properties and allowed for a fixed loading pressure. The mechanical properties of the 3D printed VeroWhite Plus material increased as the thickness increased, as demonstrated in equation 7.2.

The measured signal from the 3D printed sensor was only obtained after the sensor was assembled with the polymer adhesive, which increased the modulus of the sensor by 75% [16] and removed the error in the loading pressure caused from the modulus of the material.

In addition to the challenges caused by the mechanical properties, the screw design did not completely encapsulate the pressure sensor, and the leads on the back side of the AktivSensor, shown in Figure 7.3, were exposed to water. This created a short in the circuit, which made it impossible to generate an electrical signal measurement of the pressure change. The use of adhesive makes it possible to create a water-tight seal on the sensor cover, keeping the sensor dry when immersed into the aqueous media solution.

7.4.2 Mechanical Stability of the Sensor Diaphragm

The limiting factor in this experiment was the piezoresistive sensing diaphragm, which utilizes silicon. As stated in the results section, the pressure measured on the pressure sensor was 67 KPa. This measurement was collected just prior to the

mechanical failure. This measurement falls in accordance with the characterized pH response of the hydrogel composition used. Furthermore, the response is higher than the mechanical stability of the sensor, which is 60 KPa. The first conclusion to be drawn is that the maximum pressure on the sensor specifications sheet is already pushing the threshold of the sensor, and therefore, the pressure exerted on the sensor should neither equal nor exceed the maximum pressure listed.

According to Griffith's Fracture Theory, an initiated crack extends as the rate of release of the stored elastic strain energy exceeds the rate of energy absorption. This occurs as the formation of new surfaces with specific surface energy [17]. Fracturing of the sensing diaphragm, defined as a plate, can be defined by equation 7.2.

$$\sigma_f^2 = \left(\frac{2E\gamma}{\pi a}\right)^{\frac{1}{2}} \tag{7.2}$$

In this equation, the tensile stress is represented as σ_f, while E is the Young's modulus, γ is the surface energy, π is the numerical value for pi, and a represents the crack length. The numerical values for silicon for the variables given in equation 7.3 are provided in Table 7.2.

After solving equation 7.2 for a, the surface will crack will cause the plate to fail when the crack length reaches 1.22×10^{-15} m (see equation 7.3).

$$a = \frac{2E\gamma}{\sigma_f^2 \pi} \tag{7.3}$$

Furthermore, equation 7.3 has been rearranged to predict the required Young's modulus of a piezoresistive material that could withstand the pressure exerted on the plate by the hydrogel sample (see equation 7.4).

Table 7.2. Numerical values for silicon for use with Griffith's equation (7.1)

Variable	Value
σ_f	756.3 Pa
γ	1.986×10^{-11} J/m^2
E	60 KPa

$$E = \frac{\sigma_f^2 \pi a}{2\gamma} \tag{7.4}$$

Using the values presented in Table 7.2 and a crack length of .001 μm, the modulus required for use with the optimized hydrogel is 240 KPa. This can be achieved by increasing the thickness of the pressure sensitive plate. The supplier for the AktivSensor has stated that the thickness of the bending plate can be customized based on the application and needed pressure range.

The elastic strain energy that is stored in the plate is defined as W. According to Griffith's Fracture Theory, the stored elastic strain energy is defined by equation 7.5.

$$W = \frac{\pi \sigma^2 a^2 t}{E} \tag{7.5}$$

In this equation, all variables are as defined above, and t is defined as the plate thickness. Therefore, equation 7.5 can be used to predict the thickness needed to tolerate the swelling pressure generated by the hydrogel sample, where W is a material constant and t is the only altered value. If the original plate thickness is 10 μm, then, after solving equation 7.5 for t, the thickness should be greater than or equal to 24 μm.

7.4.3 Hydrogel Considerations

The magnitude of the hydrogel swelling response is ideal for high sensitivity to change in analyte concentration; however, as explained, the mechanical stability of the

pressure sensor used was much smaller than the swelling pressure, which resulted in a fracturing of the sensing diaphragm. This incompatibility between the sensor and hydrogel could be designed out of the assembly by increasing the degree of cross-linking of the polymer matrix. However, this would decrease the sensitivity of the optimized material utilized in this experiment.

7.5 Conclusion

The purpose of this experiment was to investigate the use of 3D printed materials for the sensor cover. While the mechanical stability of the sensing diaphragm limits the sensitivity of the sensor, experiments outlined in this chapter demonstrate that 3D printed materials could be used to manufacture inexpensive pressure sensors without interfering with the functionality of the hydrogel-based chemomechanical sensors.

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CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

8.1 Chemical Sensors

Chemical sensors are devices that provide data on the composition of a specified environment. In the case of this project, hydrogel-based chemomechanical sensors were used to provide information on the media solution within a bioreactor during its use. Two components of a chemical sensor are the signal recognition piece, which recognizes the desired analyte, and the signal transduction piece, which transduces the measured signal change into a quantifiable data. The pH-responsive hydrogel developed in this project provides the recognition component of the chemical sensors developed. The piezoresistive sensing diaphragm served as the signal transduction method. The results obtained from this project were analyzed in terms of sensitivity, selectivity, and response time, and were compared to other methods of pH detection currently used in bioreactor applications.

8.1.1 Sensitivity

To determine the sensitivity of the optimized hydrogel composition, the hydrogel was tested with fixed ionic strength and temperature. The hydrogel sample was tested in

small increments spanning between 0.01 to 0.1 over a large spectrum of pH ranges, from 6.0 to 7.4 in phosphate buffered saline. The pressure magnitude vs. the pH were plotted and analyzed, and are given in Figure 8.1.

The limit of detection represents the lowest quantity of a substance that can be detected from the absence of that substance. In this experiment, the limit of detection was calculated using equation 8.1, where s is the signal, represented as the magnitude of the response and n is the noise, represented as the difference between the highest peak and the lowest peak in the pressure response.

$$LOD = \frac{S}{N} \qquad 8.1$$

The limit of detection was calculated with the n value being equal to 11.98 and the s value being equal to 990.84 Pa. The resulting signal-to-noise ratio is 825 Pa. Since this

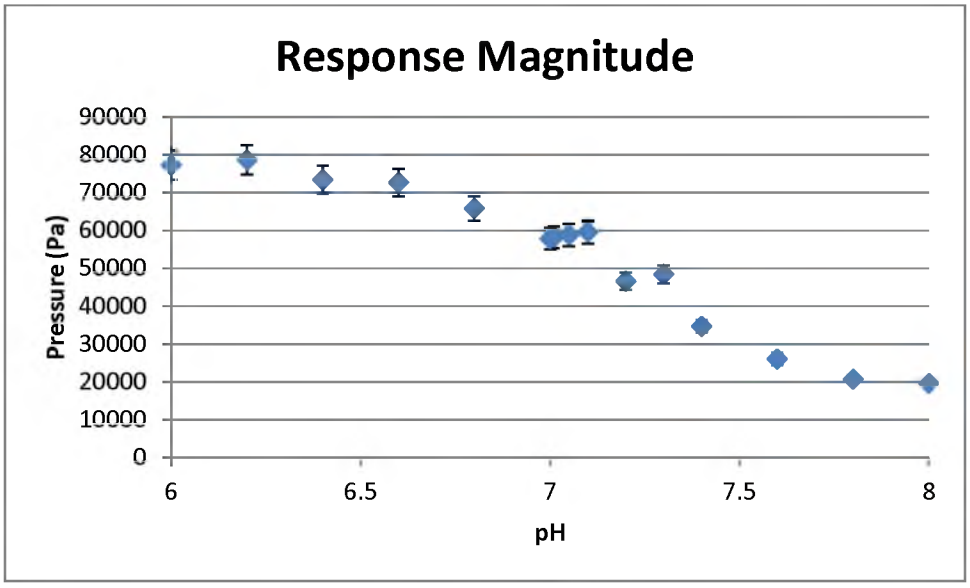

Figure 8.1. A response vs. pH plot demonstrating the sensitivity of the hydrogel composition.

number is greater than 3, the signal is strong enough to detect the signal. Furthermore, the limit of quantification is where the signal-to-noise ratio is greater than 10. In this case, the ratio is well over 10, and therefore, detecting between a pH of 0.05 is well within the capacity of this hydrogel to measure reasonable differences at a higher resolution than 0.05 pH values. Furthermore, the limit of detection is equal to three times the noise. In this experiment, the noise was calculated at 11.98 Pa. When this value is multiplied by three, the resulting value is 35.94 Pa, which means that the hydrogel used in this project can detect pH changes that result in greater than 35.94 Pa.

The sensitivity of the optimized hydrogel is characterized by the slope of the line of the linear portion of the graph. The slope is calculated as 4.8, and the corresponding sensitivity is valued at 4.8 KPa/pH.

8.1.2 Selectivity

The selectivity of a sensor is the ability of a sensor to distinguish one chemical from another in a system. The selectivity is determined by calculating the selectivity coefficient represented in equation 8.2.

$$SC = \frac{Response\ Magnitude\ of\ Other\ Analytes}{Response\ Magnitude\ of\ Desired\ Analyte} \quad 8.2$$

The hydrogel composition used in this project was tested to determine the response to changes in pH as well as other chemical analytes, as indicated in Chapter 3. These responses include changes in ionic strength as well as changes in the amount of ions in solution. The specific ions used in testing include HCl and $Ca(OH)_2$. The hydrogel response between pH values of 7.2 and 7.4 is 3.6 KPa. The response magnitude to other analytes are: 0.001 M $Ca(OH)_2$ (0.58 KPa), 0.001 M HCl (0.80 KPa), and ionic

strength between 155 mM and 165 mM (1.60 KPa). The selectivity coefficient for HCl is 0.22, for Ca(OH)2 is 0.16, and for the ionic strength response is 0.44. This value indicates that the hydrogel is most selective to the change in pH, but of the responses given, there is a certain ratio of the response that comes from other analytes.

8.1.3 Figures of Merit and Comparison to Other pH Monitoring Methods

The results of the determination of the sensitivity and selectivity were compared to figures of merit in other methods used in bioreactor monitoring. The data presented in Table 8.1 compare the two methods currently used, optical and electrode, to hydrogel methods.

Table 8.1. A table representing the figures of merit of other pH sensors used in bioreactor applications

Sensor Type	Sensitivity	Selectivity	Response Time
PreSens pH Sensor			
Optical [6]	pH of 5.5 to 8.5, 0.05 sensitivity	Sensitive to ionic strength and temperature	30 seconds
Medorex pH Electrode			
Electrode [60]	pH 1.0 to 13.0, 0.01 sensitivity	Sensitive to ionic strength and temperature	Less than 30 seconds
pH-Responsive Hydrogel Chemomechanical Sensor			
Hydrogel	pH of 5.0 to 8.0, >0.01 sensitivity	Sensitive to ionic strength	Less than 30 seconds for >0.05 pH change, and nearly 1 minute for 0.2 pH change

For optical methods, the main disadvantages include the degradation of the signal over time due to photobleaching. In addition, the pH change measured by the device is lower than the pH change of the actual system [6]. Optical methods are also dependent upon the pH range of the detection component used. Jeevarajan et al. used phenol red to detect changes in pH. They found that the total range of phenol red is 2 pH levels with a 0.05 sensitivity range, with some variation. Their research provides a device that is useful under specific applications as specified by NASA for disposable bioreactors.

Methods utilizing pH electrodes have been in use for over 100 years as a method for detecting changes in pH. The value of a pH electrode is that it will respond across the entire pH range, from 1-14, with 0.01 sensitivity. However, there is a steady baseline drift over time that increases as the temperature increases. Furthermore, the life of the electrode is also dependent upon the temperature of use, for example, the life of electrodes used in 25 ^{o}C is an average of 6 months, while electrodes used in 100 ^{o}C will last for an average of 1 month. While the lifetime of these electrodes is stated, the baseline consistently drifts and must be recalibrated in 25 ^{o}C after the baseline has drifted by 15 mV. Other disadvantages to pH electrodes include the challenges with detecting changes in pH in solutions of low conductivity. Electrodes also operate optimally in solutions of >40% aqueous media to avoid solvent effects. The last mentioned disadvantage is that as the temperature increases, the response time also increases.

8.2 Advantages and Disadvantages of pH-Responsive Chemomechanical Sensors

Hydrogels are not a perfect method for detecting the change in pH, and they have many of the same limitations with respect to selectivity; however, hydrogels have a long shelf life and function without a baseline drift or the need for calibration. Furthermore, as specified, the limit of detection for hydrogels is 35.94 Pa, which means the signal to noise ratio is small, which means hydrogels can be used to detect small fluctuations in pH with a fast response time.

pH-responsive hydrogels have other advantages in addition to those compared in Table 8.1. Hydrogels are sterilizable with gamma sterilization, unlike optical and enzymatic pH sensors. Furthermore, enzymatic pH sensors are reactive with the contents of bioreactors and cannot be used for bioreactor applications. Because of the storage and operational stability outlined in Chapter 5, hydrogels can be stored and used for extended periods of time. Hydrogels with fluorescent molecules and other optical methods that utilize fluorescence begin to lose sensitivity over time due to photobleaching.

8.3 Thesis Objectives and Conclusions

The motivation of this project was to create a continuous pH monitoring device that could measure systemic changes in pH. In the biomedical industry, such a device could be used to help physicians make a rapid diagnosis and treat patients for shock and acidosis before further risking their lives for other major treatments that may be necessary after injury [1-3]. In addition to systemic pH monitoring, a continuous pH monitoring device would be useful in the biotechnology and tissue engineering industries.

Bioreactors are enclosed systems that provide optimal environments for the culture of biological structures. A slight change in pH could foul the contents of a bioreactor [4-14].

In biomedical applications, the only current pH monitoring technology available does not incorporate the ability of continuous monitoring. Rather, it requires a patient to rinse their mouth and test the saliva with color changing paper. In bioreactor applications, there are more methods available, including optical measurements, glass electrodes, and ionic sensitive field effect transistors. These methods present challenges, including loss of mechanical stability and reactivity with the contents of the bioreactor, which limit the ability of the current technology to satisfy the needs of the industry. Furthermore, pH is currently measured after removing a sample of media solution from the bioreactor, which introduces contaminants.

Methods for continuous monitoring of pH conditions of systems are needed both in the healthcare and bioreactor industries. One of the challenges of current continuous pH monitoring devices is that failures in sensor performance lie in the irregularity of analytical performance. To meet the demands of continuous monitoring, pH sensors must be reliable. They must also be self-contained devices that provide quantifiable information in direct contact with the media solution; must function reliably for hours without physical, chemical, or signal degradation; and must have a fast response and high sensitivity to small changes in the local environment [15-59]. Table 8.1 outlines the objectives and conclusions of this thesis project.

Table 8.2. Objectives and conclusions of hydrogel-based chemomechanical sensors

Objectives	Conclusions
Characterize the response of HEMA hydrogels: the response time, the response to small changes in pH, and the response to other analytes	Hydrogels with a HEMA back bone structure and DMA sensing groups can be used as stimuli responsive materials. The experiments outlined in this chapter demonstrate the response of hydrogel samples to changes in pH, ionic strength, and changes in ion concentration. Due to the data presented here, hydrogels prove that they are multi-analyte sensitive materials that can be tested within normal ranges of systemic physiological conditions as well as in bioreactor applications. In addition, the response time for hydrogels is not ideal for continuous systemic monitoring; therefore, future research projects will be designed to optimize the response time.
Optimize the response of HEMA and HPMA hydrogels: determine the function of each monomer, determine the ideal composition/ratio of monomers, determine the effect of thickness on the response time	The components used in synthesizing hydrogel samples were analyzed in this chapter to determine their effect on the optimization of the hydrogel stimuli response. The degree of cross-linking and polymerization is directly impacted by the concentrations of cross-linking monomers or photo initiator. Ethylene glycol is more than just a solvent for the monomers used in synthesizing hydrogels; it is also a necessary component that creates ideal mechanical properties for testing hydrogels in the pressure sensor. The ratio of DMA to backbone monomers (HEMA or HPMA) will directly affect the stimuli response time and sensitivity. The sensitivity of the hydrogel matrix will decrease as the response time decreases. Therefore, optimization of the stimuli response must consider both the composition and thickness of the hydrogel sample. The response time of a pH-responsive hydrogel decreases with decreasing thickness. The dependence on thickness is stronger than quadratic for thick gels, but much weaker than this for gels thinner than 100 μm. The magnitude of the hydrogel response in a chemomechanical sensor also decreases as the thickness decreases, but the dependence is weaker than linear. Therefore, sensor response time can be reduced significantly by using thin hydrogels without compromise of the ability to detect small pH changes.

Table 8.2 continued. Objectives and conclusions of hydrogel-based chemomechanical sensors

Objectives	Conclusions
Determine the shelf-life of HEMA hydrogels: examine the change in response over time and examine the response over continuous cycles	The experiments performed in this project were designed to determine if a hydrogel sample could be stored for an extended period of time and to determine if a hydrogel sample could be tested continuously. Samples taken from a hydrogel monolith were tested immediately after synthesis and after 9 and 18 months of storage at ambient conditions. The hydrogel responded in the same manner for all three of the tests. The hydrogel samples were also tested continuously through repeated cycles to determine the effects of the hydrogel after prolonged testing. The hydrogel responded with a similar magnitude and response time throughout the continuous testing with no significant decrease in sensitivity. The results of these tests demonstrate that hydrogels can be used after being stored for an extended period of time and can be used in continuous cycle testing.
Synthesize a hydrogel in situ in a prefabricated sensor and determine effects of in situ synthesis and shipping on the pH response of the hydrogel	Comparison has been made between two different designs of chemomechanical sensors: the M-Biotech design and the Boss design. The M-Biotech design is more convenient for reuse with different hydrogel samples. On the other hand, the Boss sensor can be used with thinner hydrogel samples to give faster sensor response kinetics without loss of sensitivity. In addition, the experimental results obtained in this project demonstrate that hydrogels can be synthesized, dried, and then rehydrated after a period of time without losing their ability to respond to environmental stimuli.
Develop cost effective methods for manufacturing hydrogel sensors through 3D printing	The purpose of this experiment was to investigate the use of 3D printed materials for the sensor cover. While the mechanical stability of the sensing diaphragm limits the sensitivity of the sensor, experiments outlined in this chapter demonstrate that 3D printed materials could be used to manufacture inexpensive pressure sensors without interfering with the functionality of the hydrogel-based chemomechanical sensors.

8.4 Significance of Research

8.4.1 Hydrogel Stimuli Response Characterization

Initially, a stimuli responsive hydrogel composition was selected for testing. The hydrogel was synthesized, conditioned, and tested to determine the response to changes in different analyte concentrations, including pH, ionic strength, glucose, and chloride ions. Initial weighing tests demonstrated a varied response to different solutions with a fixed pH value. Upon further investigation into the swelling response, it was determined that the degree of swelling in response to different pH buffers was a result of changes in ionic strength across buffered solutions. This was proven in the experiments of measuring the response to changes in ionic strength concentration. Therefore, hydrogels are multi-analyte sensitive and the degree of swelling is a function of both the composition of the hydrogel and the composition of the surrounding media solution.

In addition to determining that hydrogels will respond to changes in pH and ionic strength conditions, hydrogels in this chapter were tested to determine the cross-sensitivity response to other analytes to which hydrogels have a known response. HEMA hydrogels with a DMA sensing group do not respond to changes in glucose concentration, but they do respond to changes in ion concentration. Both of these results were predicted, but data from experimentation validated the prediction. Under fixed ionic strength and pH conditions, HEMA hydrogels could be used in future applications to monitor small fluctuations in ion concentrations.

8.4.2 Optimization of Swelling Response

Initial investigations into the swelling response of HEMA hydrogels revealed that the response time was longer than would be ideal for continuous monitoring applications. Because of this phenomenon, a series of experiments was performed in which the actual function of each monomer used for hydrogel synthesis was tested. Changes were made in hydrogel composition based on the function of the monomers used. After determining the function, hydrogels of modified composition, porosity, and thickness were tested to optimize the hydrogel response time.

Several key features were discovered during these experiments. As the amount of DMA increases, the sensitivity increases, but the response time also increases. Furthermore, as the amount of TEGDMA increases, the sensitivity decreases, and the response time decreases. Therefore, there are tradeoffs that exist as the hydrogel composition is modified to meet the desired specifications. While this is the case, the degree of swelling can be manipulated and predicted based on the results of these experiments.

During this series of experiments, the hydrogel response time was optimized by decreasing the thickness of the hydrogel sample. Hydrogels with a thickness of 400 μm have an excessively long response time, despite the composition. The response time of the varying thicknesses demonstrated a nonlinear, almost second power, relationship with regard to the response time versus the thickness of the gel. The result of experimentation is that there is no significant difference between hydrogels of 50 μm in thickness compared to hydrogels of 100 μm in thickness. Furthermore, the hydrogel response magnitude also decreases, while not to the same degree, as the thickness decreases.

Therefore, thickness should not decrease beyond the point where the signal cannot be separated from the noise.

8.4.3 Study of Hydrogel Stability

In this series of experiments, the initial composition of HEMA hydrogels was synthesized and tested to determine the storage stability of hydrogels as well as the operational stability, which is defined as the ability of hydrogels to continue their swelling response after repeated testing.

After the initial hydrogel synthesis, the swelling response was characterized. The same hydrogel monolith was placed on a shelf in the lab and tested again after 9 months and again after 18 months. At each time interval, the hydrogel demonstrated the same response. This demonstrated that hydrogels can be stored under ambient conditions for extended periods of time.

In addition to testing the hydrogel to determine the shelf life, the same hydrogel monolith was tested for repeated cycles to determine if a hydrogel sample would continue to respond with the same magnitude during continuous monitoring. A hydrogel sample was tested for more than 300 cycles, and the magnitude of the response remained constant. Because of this experiment, the swelling response is not only reversible, but the magnitude of the response remains constant. Therefore, the operational stability of hydrogel samples has demonstrated that hydrogel-based sensors could be used in continuous sensing applications.

One key discovery in this experiment is that initially, the deswelling response magnitude was higher than the swelling response magnitude. This was observed in the

analysis of the time versus pressure plot. This experiment was validated and in each case, the hydrogel swelling response approached equilibrium with the deswelling response after 30 cycles. Therefore, hydrogel-based sensors must be conditioned for at least 30 cycles before calibration can occur. This phenomenon can be explained by considering the polymer matrix. After the hydrogel is synthesized, there may be unreacted monomers confined within the network. As the hydrogel swells and stretches, the unreacted monomers and shorter polymer chains are able to diffuse through the matrix. With each swelling and deswelling cycle, the unreacted monomers move closer to the interface and eventually leave the matrix. This could explain why both the response time and the magnitude of the deswelling response was initially greater than the swelling responses.

8.4.4 Device Miniaturization Applications

A group in Germany collaborated on a set of experiments. The investigator sent a piezoelectric microsensor with a mechanically attached "Boss" component. The surface of the sensing diaphragm was treated with an adhesion promoter, and a hydrogel sample was polymerized on the surface of the sensor. The sensor was shipped to Germany for testing.

To ensure the hydrogel would perform as it had in the lab, a monolith was synthesized and a series of transportation experiments were performed prior to hydration. After simulating transportation conditions, the hydrogel was hydrated, washed, conditioned under the normal procedures, and tested in the pressure sensor. The hydrogel

responded with the same swelling behavior, which indicated that shipping conditions would not have adverse effects on the stimuli response of the hydrogel.

The microsensor was stored on the shelf in Germany for 6 months prior to testing, and was then tested under the same conditions. The results were compatible with previous results obtained. The thickness of hydrogel samples tested in the lab was 400 μm, with a very slow response time. The hydrogel sample loaded into the microsensor was 50 μm with a response time that fell in accordance with experimental results that demonstrate a decreased response time for thinner hydrogels.

This series of experiments proved that hydrogel samples can withstand transportation without special attention. Furthermore, data from other experiments was validated regarding the thickness of hydrogel samples.

8.4.5 3D Printed Sensor

In the first series of experiments, a macro sensor was used to characterize the stimuli response. The major limitations to the measurement tools used were the high cost and long production time of the piezoresistive sensors that are commercially available. In addition, the sensor cover was a machined part that was also costly and had a long production time. An inexpensive off-the-shelf pressure sensor was used in combination with 3D printed parts to address the high cost and long production time [15]. The assembly was used to compare the results among sensor assemblies to determine the loss of effectiveness with using more cost-effective parts.

The 3D printed materials did not have the desired mechanical properties, and were therefore redesigned to better meet the requirements. As a result, 3D printed

material is not ideal for this application, but molding and casting with a material with a higher modulus may demonstrate a more effective assembly. In addition to the 3D printed material, the sensing diaphragm did not have the mechanical stability to measure the pH-response of the hydrogel. Because of this, a different sensor should be considered. While the mechanical stability of the sensing diaphragm limits the sensitivity of the sensor, experiments performed demonstrate an equivalent pH response, further demonstrating that 3D printed materials could be used to manufacture inexpensive, disposable pressure sensors without interfering with the functionality of the hydrogel-based chemomechanical sensors. The Boss sensor assembly is better because it allows for thinner hydrogel samples.

8.5 Future Directions

The data presented in this dissertation demonstrate the ability of hydrogels to be used in hydrogel-based sensor applications. However, future experimentation and design are needed to further meet the needs of the industry. The sensors used in this project were all purchased from companies as off-the-shelf options. Future work needs to be done in which the piezoresistive sensor is fabricated on a microscale to ensure the sensor is designed to meet the ideal operational specifications of the hydrogel, including mechanical stability. As demonstrated, hydrogel swelling can occur with a high magnitude response. Optimized hydrogels have a decreased response time and magnitude, but the sensitivity of the hydrogel should not be compromised to maintain the integrity of the sensing diaphragm. Microfabrication of the pressure sensor could also help meet the size parameters of industrial applications.

Hydrogels used in this project were tested in the majority of cases with sensors that could be reused. In both biomedical and bioreactor applications, sensors will be manufactured at a low cost and will have no need to be reused. Therefore, future experimental approaches could focus on an integrated hydrogel-based sensor where the hydrogel sample is housed more permanently inside the sensor cavity.

8.6 References

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